INTRINSIC MARKERS IN AVIAN POPULATIONS:

EXPLORATIONS IN STABLE ISOTOPES, CONTAMINANTS, AND GENETICS

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ABSTRACT

This research outlines the diversity of questions that intrinsic markers have the potential to answer and demonstrates some of these marker's limitations and successes. To test the working hypothesis that feathers grown on different continents have significantly different stable isotope ratios in commonly used markers, I analyzed stable isotopes in two generations of feathers from three species of birds that breed at high latitudes and winter on different continents. As expected, significant differences in stable isotope ratios were detected between summer- and winter-grown feathers in both plover species (*Pluvialis fulva* and *P. domininca*). However, no differences were found between the two groups of winter-grown plover feathers, despite being grown on different continents. Similarly, no differences were detected in isotope values between summer- and winter-grown feathers in northern wheatears (*Oenanthe oenanthe*). Large variances in isotope ratios limited the percentage of feathers correctly assigned to their origins to 41%.

Atmospheric transport has been identified as the source of pollutants in several arctic ecosystems and has the potential to severely impact high-latitude populations. To determine whether long-range atmospheric transport, point sources, or migratory prey were sources of contaminants in the North Pacific, birds from two trophic levels were sampled across the longitudinal transect of the Aleutian Archipelago. Carbon isotope ratios differed among islands, thereby linking birds to island food webs and ruling out contaminant transfer through migratory prey. Patterns in some PCB congeners indicated

local point sources, but significant west-to-east declines in contaminant concentrations for the majority of detected organochlorines provided evidence of long-range transport.

Linking individuals to source populations using intrinsic markers has only been successful at broad scales. To determine whether increased resolution among populations could be achieved by merging multiple intrinsic marker classes, a new analytical procedure was developed. Discrete and continuous markers were combined to evaluate a Bayesian method of assignment across marker classes. For three datasets, two real and one simulated, the percentage of individuals assigned to correct source populations increased with the addition of markers and marker classes. In all cases, the maximum number of individuals was correctly assigned when all marker classes were combined.

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GENERAL INTRODUCTION

Large- and small-scale movements of individuals and populations are difficult to monitor. Such daily and seasonal movements have left large gaps in our understanding of animal behavior, the dynamics of populations, and, at times, confounded the interpretation of contaminants studies. Technological advances have led to the ability to quantify intrinsic markers for tracking individuals and delineating populations. The ability to correctly assign individuals to populations of origin will increase our effectiveness in studying animal migrations, sources of environmental contamination, and the dynamics of populations.

Several stable isotopes exhibit geographic variation and are transferred to consumer organisms in a predictable manner. Stable isotopes are incorporated into keratin tissues, such as feathers, only during growth, and thus they provide a geographic signal of where these tissues were grown. Similarly, inherited traits such as genetic markers may provide signatures at the population level. Singly, an intrinsic marker is often too "fuzzy" to delineate populations or regions of origin. Combining these marker classes in analyses has the potential to increase resolution among populations.

Long-distance foraging or consumption of migratory prey species can expose non-migratory animals to distant contaminants and confound interpretation of the data. In this research, I used geographically distinct carbon stable isotopes to link cormorants to local food webs, thereby removing the confounding factor of migratory prey as a contaminant source. In another study, I used geographically distinct multiple stable isotopes incorporated in feathers of three migratory species that breed in Alaska to link

breeding grounds to wintering areas on three different continents. Although some insights were gained from this study, geographic signals were not resolute enough to link broad geographic regions using just this one class of intrinsic marker.

Finally, to test whether resolution among populations would increase by combining multiple classes of markers, I merged stable isotopes, morphological characters, and genetic data to form datasets with discrete and continuous marker classes. Bayesian methods were used to determine the probability of membership among reference populations, because analyses with classical statistical methods are impractical for datasets that combine discrete and continuous data. This method for combining marker classes for analyses has broad applicability and should prove useful to ecologists and wildlife managers.

In summary, this research was an exploration of ecological questions that potentially can be answered using the development of intrinsic markers and new methods to analyze associated data. In the following chapters, I show that the power of intrinsic markers as analytical tools is directly related to the distinctiveness of the marker(s) and the scale of the question(s) being asked.

CHAPTER ONE

HOW USEFUL ARE FEATHER STABLE ISOTOPES FOR DETERMINING ORIGINS IN INTERCONTINENTAL MIGRATORY BIRDS?

Abstract

The application of stable isotopes to link breeding and wintering areas in migrating animals is having a profound impact on biology. Geographic origins of several bird species have been inferred from wintering birds using geographically distinct isotopes in their tissues. Linking mobile individuals to geographic regions critical in their life cycles is an important step for understanding migration systems and populations and also for effective conservation and management. Yet the ability to accurately assign individuals to areas of origin based on multiple stable isotopes has received little attention. I analyzed stable isotopes in two generations of feathers from three species of birds that breed at high latitudes and winter on different continents to test the hypothesis that feathers grown on different continents will have significantly different stable isotope ratios in commonly used markers (δD, $\delta^{13}C$, and $\delta^{15}N$). I also hypothesized that these markers would be distinct enough to permit accurate assignment of feathers to known areas of origin. In this study, all three species, Pacific and American golden plovers (*Pluvialis fulva* and *P. dominica*) and northern wheatears (*Oenanthe oenanthe*), molt both on Alaska breeding areas and on their wintering areas in the South Pacific and Asia, South America, and Africa, respectively. I found significant differences in stable isotope signatures between summer- and winter-grown feathers in the plovers, but detected no

differences between the two groups of winter-grown plover feathers, despite being grown on different continents. Similarly, no differences were detected in isotope values between summer and winter feathers in wheatears. I introduce an "assignment with exclusion" method to determine the accuracy of assigning plover feathers to correct groups of geographic origin based on multiple stable isotopes but obtained an overall accuracy of only 41%. This study demonstrates that, while useful in many applications, stable isotope signatures in feathers may not be distinctive enough in all cases to permit accurate seasonal tracking of either individuals or populations.

Introduction

Understanding migratory species requires knowledge of seasonal movements. Historically, these movements have been difficult to monitor in many species due to a general lack of population-level markers. However, recent scientific advances have led to the development of intrinsic markers for tracking individuals and, in aggregate, populations. Recently, geographically distinct stable isotopes were used to infer breeding origins in birds from Neotropical wintering areas (Hobson and Wassenaar 1997; Rubenstein et al. 2002) and to delineate breeding and wintering areas (Chamberlain et al. 1997; Rubenstein et al. 2002). This research has led to a proliferation in the application of stable isotopes for linking regions used by migratory animals (Hobson 1999).

The potential for using stable isotopes as markers to study migratory animals relies on the assumption that unique signatures of stable isotopes incorporated into tissues from an animal's diet can be correctly linked to areas of origin. In keratin tissues, such as feathers or hair, isotopes are incorporated only during growth (Mizutani et al. 1990) and

thus provide a geographic signal of where these tissues were grown. Geographically distinct isotope signatures have the potential to link wintering or migrating birds to geographic origins of molt throughout the annual cycle. In birds that retain more than one generation of feathers per cycle (i.e., different feathers are molted in different regions), geographic locations of molt (e.g., breeding and wintering areas) may be identified if feather isotope signatures are geographically distinct. Several stable isotopes show distinct geographic patterns, but carbon ($\delta^{13}C$) and hydrogen (δD) show considerable variation with respect to latitude, elevation, and climate (Dansgaard 1964; Korner et al. 1991; Hobson 1999; Rubenstein et al. 2002; Graves et al. 2002). For example, δD tends to become progressively depleted (more negative) away from the equator, with some of the most depleted global values occurring in Alaska and northern Canada (International Atomic Energy Agency 2001), and it thus may serve as a latitudinal signature. Carbon isotopes vary with respect to C_3 and C_4 vegetation and are generally distinct between arid and moist climates (Korner et al. 1991). Although $\delta^{15}N$ has been primarily used to designate trophic levels in food webs, broad scale geographic patterns have been identified (DeNiro and Epstein 1981). Combining multiple isotopic markers that vary geographically but independently of each other, may substantially improve discrimination among populations and permit the assignment of individuals of unknown origin to populations or geographic regions.

Assignment of individuals to populations or areas of origin in this field has thus far been accomplished rather incidentally through association by matching individuals to the geographic signature of highest similarity. A drawback to this and other assignment

methods is that a probable source of the individual being classified is always designated, even if the true source is not represented in the reference set (Cornuet et al. 1999). Therefore, there can be little confidence that the correct source has been identified. Here I adopt an "assignment with exclusion" method from population genetics, which uses an exclusion criterion to reject unlikely sources. This new assignment method was used to evaluate the potential of using multiple stable isotopes to assign feathers to location of origin. I used a natural system among three species of intercontinental migrants that breed in Alaska, and in each species I sampled two generations of feathers, each grown on different continents. I hypothesized that δD and $\delta^{13}C$ in feathers grown on Alaska breeding areas would reflect high-latitude environmental signatures and that they would differ markedly from feathers grown the previous season on low-latitude wintering grounds on different continents. Finally, I evaluated the usefulness of multiple feather stable isotopes to delineate groups and link birds of known breeding origin to wintering locations known at the continental scale.

Materials and Methods

Focal Species. American and Pacific golden plovers (*Pluvialis dominica* and *P. fulva*) are sibling species that breed sympatrically in western Alaska and occupy geographically distinct wintering areas (American Ornithologists' Union 1998). *P. dominica* migrates to wintering grounds in South America, and *P. fulva* winters in Hawaii, Asia, and Australia (Fig. 1.1). Both species molt into dark breeding feathers ventrally (alternate plumage) on their wintering grounds, with some individuals completing this molt en route to breeding areas (Johnson and Connors 1996). Pale,

summer-grown, ventral body feathers (basic plumage) begin replacing winter-grown plumage during incubation (Johnson and Connors 1996). I sampled both types of feathers from individuals collected in 1998 and 2000 on breeding territories along the Nome-Taylor Highway on the Seward Peninsula, Alaska. Alternate, winter-grown breast feathers were considerably darker and easily distinguished from basic, summer-grown breast feathers that were newly grown and almost white (Fig. 1.2). To ensure that all basic feathers were Alaska-grown, only fresh (usually in sheath), unworn, pale feathers were used in this study. I sampled feathers from adult birds collected on their breeding territories within a 100-km area to ensure minimal within- and between-species variation in isotopic signatures of samples from the breeding grounds.

Northern wheatears (*Oenanthe oenanthe*) were also used in this study. Adults of Alaska populations replace flight feathers on their breeding grounds during a complete prebasic molt prior to fall migration (Kren and Zoerb 2000). A partial alternate molt, which includes back feathers, occurs primarily on African wintering grounds (Kren and Zoerb 2000). I sampled both feather types from northern wheatears collected on breeding grounds during the breeding season along the Nome-Teller Highway on the Seward Peninsula and Eagle Summit, Alaska. Flight feathers were secondaries and were sampled based on worn appearance. This criterion did not allow sampling of the same feather in all cases (e.g., secondary 1), because in a few instances feathers were missing or molting, but this sampling did ensure that each flight feather was grown on Alaska breeding areas the previous season. Care was taken to check for evenness of wear among secondaries to avoid the possibility of sampling a feather grown elsewhere through adventitious molt

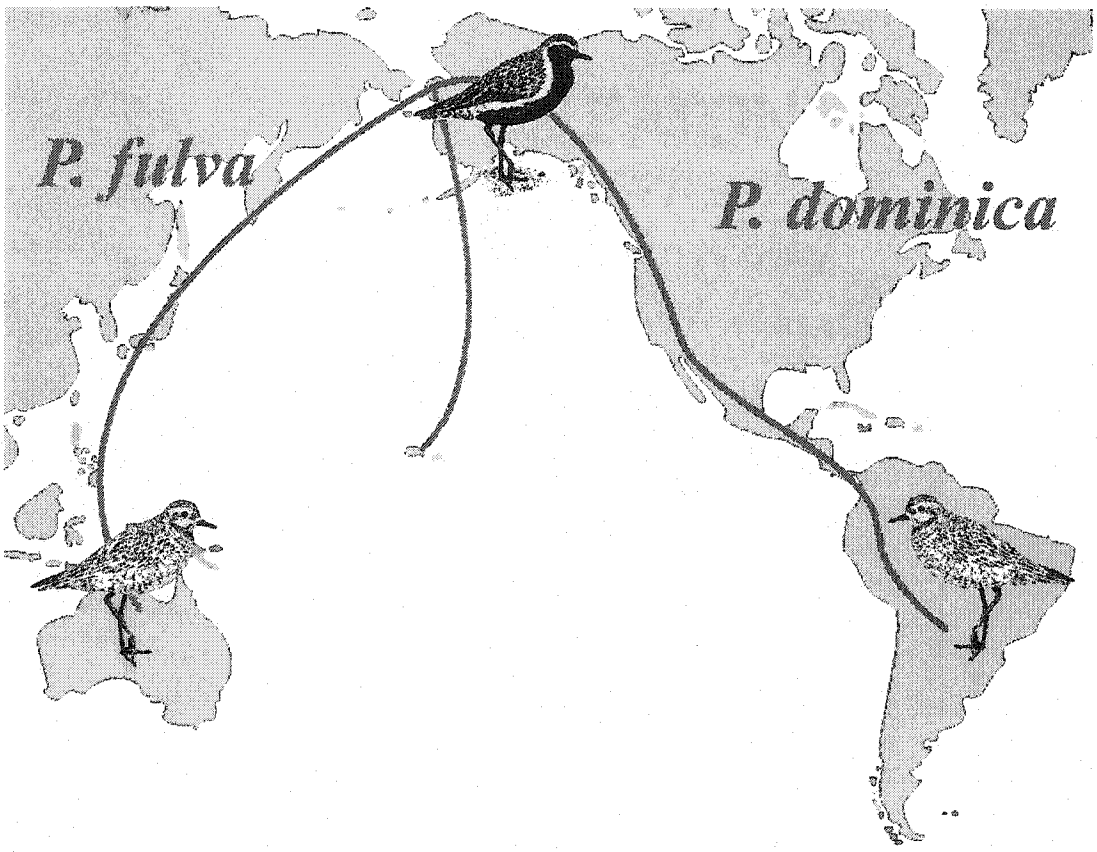

Fig. 1.1. Breeding and wintering regions and migration routes of some populations of *Pluvialis dominica* and *P. fulva*. All plovers used in this study were from breeding grounds on the Seward Peninsula, Alaska.

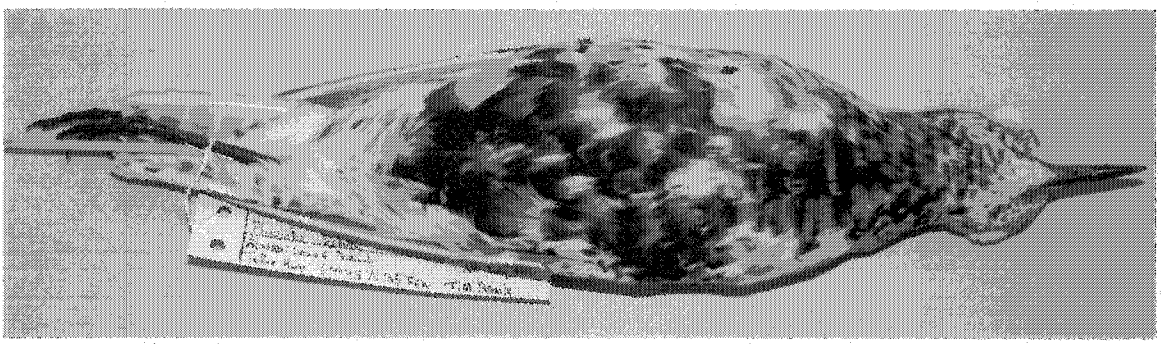

Fig. 1.2. *Pluvialis fulva* collected on Alaska breeding grounds showing dark alternate feathers and newly-molted, pale basic feathers.

(unscheduled single-feather replacement).

Isotopic Analysis. Body feathers were removed from study skins with scissors at the skin surface (voucher specimens archived at the University of Alaska Museum). Flight feathers were clipped between the vane and the calamus. Feathers were rinsed to remove any dirt or surface oils and allowed to dry. Samples were weighed (1 - 1.5 mg) into tin cups and analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the mass-spectrometry facility, University of Alaska Fairbanks, using a Europa 20/20 continuous flow isotope ratio mass-spectrometer; δD was analyzed according to Wassenaar and Hobson (2000a) at the Stable Isotope Hydrology and Ecology Laboratory, National Water Research Institute, Environment Canada. Isotopic ratios are reported as per mil (‰) deviation from the standard as defined by:

$$\delta X=([R_{sample} / R_{standard}] - 1) \times 1000, \quad (1)$$

where X is D, ^{13}C, or ^{15}N, and R is the ratio D/H^1, $^{13}C/^{12}C$, or $^{15}N/^{14}N$. Standards are Vienna standard mean ocean water (D), PeeDee Belemnite (C), and atmospheric N_2 (N). All δD values are reported as nonexchangeable H according to equations in Hobson et al. (2001).

Data Analysis. Plover feathers differed by species and season of growth, which were represented as two class variable (species and season) in analyses. Isotope values did not differ by either sex or year in any class for any isotope (Mann-Whitney $P > 0.05$); subsequent analyses were performed with year and sex pooled. Transformations failed to normalize data that exhibited non-normal distributions (Shapiro-Wilk; Table 1.1), but were homoscedastic ($P > 0.05$, Bartlett's Test). Because general linear models are

Table 1.1. Results of Shapiro-Wilk test for normality of stable isotope values in summer- and winter-grown feathers of *Pluvialis* plovers and northern wheatears (*Oenanthe oenanthe*) from Alaska.

		W- Statistic		
Species	Season	δD	$\delta^{13}C$	$\delta^{15}N$
Pluvialis dominica	Summer	0.744*	0.974	0.794*
P. dominica	Winter	0.741*	0.771*	0.891
P. fulva	Summer	0.632*	0.883	0.899
P. fulva	Winter	0.736*	0.524*	0.819*
Oenanthe oenanthe	Summer	0.861	0.822*	0.809*
O. oenanthe	Winter	0.942	0.497*	0.776*

* Data were not normally distributed within the sample, $P < 0.05$

generally robust for homoscedastic, non-normally distributed data (Sokal and Rohlf 1995), parametric tests were used in the analyses. A multivariate ANOVA (analysis of variance) design with species and season as class variables and stable isotope ratios as the response variables was used to test for differences among species, season, and the interaction (species × season). If differences were detected for class variables in multivariate analyses, univariate comparisons were performed with an ANOVA (analysis of variance) for that class.

I used discriminant analyses as an assignment method and placed an exclusion threshold of 80% or higher on the posterior probabilities for accepting group membership. Individuals with posterior probabilities of lower than 80% were excluded as probable members of a group because of an unacceptably low confidence that this group was the true source.

Northern wheatear feathers represented two classes (adult summer and adult winter) and were analyzed using parametric and non-parametric methods (Shapiro-Wilk; Table 1.1). Isotope ratios in summer- and winter-grown feathers were compared using a Wilcoxon signed ranks test for $\delta^{13}C$ and $\delta^{15}N$. A *t*-test was used to compare δD values. Lack of differences between feather types precluded further analyses in this species. All statistical analyses were performed on the Statistical Analysis System (1999, version 8e; Cary, NC).

Results

Plovers. Multivariate analyses (with δD, $\delta^{13}C$, and $\delta^{15}N$ values combined; Table 1.2) detected no significant differences between species ($F_{1,23} = 1.12$, $P = 0.36$; Fig 1.3),

Table 1.2. Stable isotope values (‰) obtained from summer- and winter-grown ventral body feathers of *Pluvialis* plovers from the Seward Peninsula, Alaska.

			$\delta D^{\dagger}$			$\delta^{13}C$			$\delta^{15}N$	
Species	Season	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD
Pluvialis dominica	Summer	5	-129	69	7	-20.51	2.27	7	5.57	2.62
P. dominica	Winter	5	-9	6	7	-17.00	1.96	7	9.72	1.76
P. fulva	Summer	9	-59	19	9	-20.93	2.60	9	6.13	1.90
P. fulva	Winter	8	-24	48	9	-18.40	3.19	9	9.24	1.24

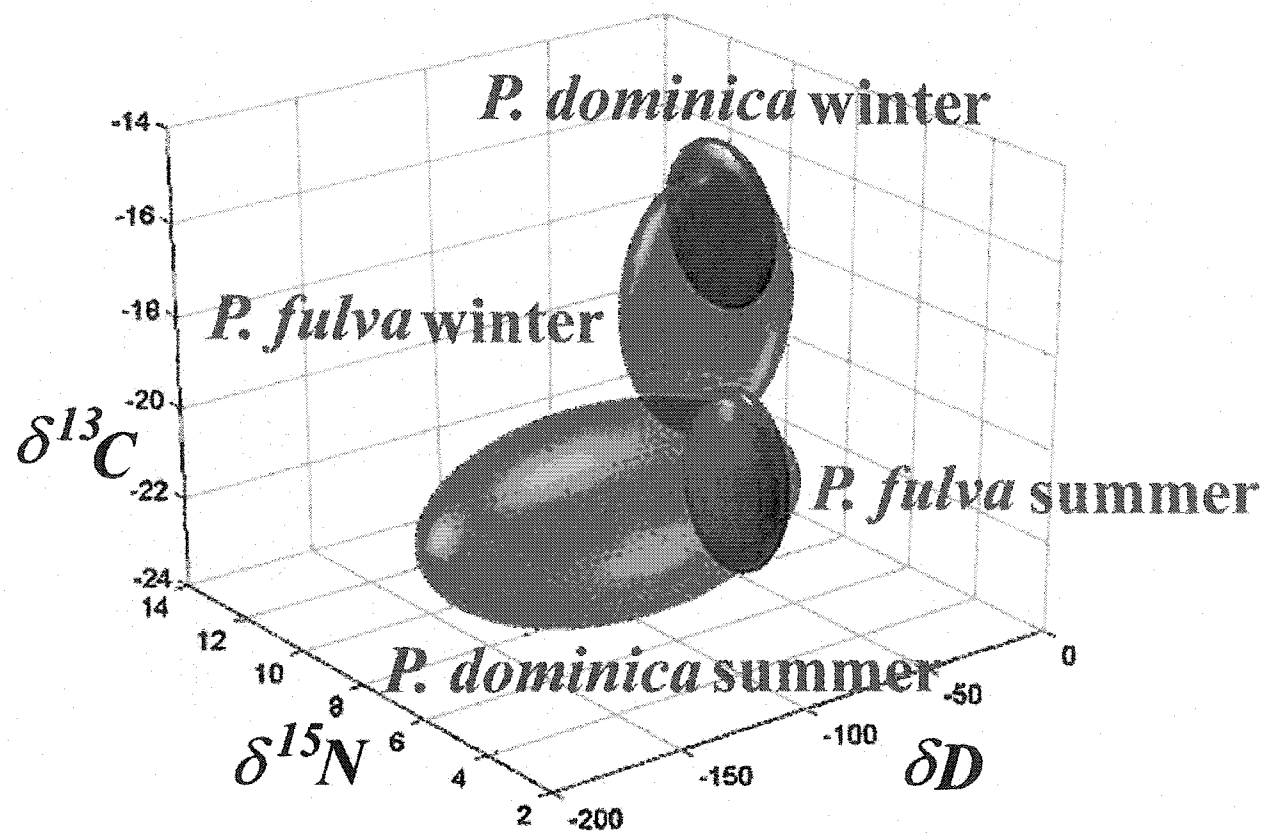

Fig. 1.3. Stable isotope ratios (95% confidence ellipses; sample sizes provided in Table 1.2) in summer-grown and winter-grown feathers of *Pluvialis fulva* (blue) and *P. dominica* (red).

but did detect significant differences between season ($F_{1,23} = 14.41$, $P < 0.01$; Fig 1.3). The species × season interaction was not significant ($F_{1,23} = 2.34$, $P = 0.10$).

Despite the considerable variation in δD values for Alaska-grown feathers (δD = -175‰ to -62‰; Table 1.2), significant differences were detected in δD values between season ($F_{1,26} = 17.59$, $P < 0.01$). Differences also existed between season for $\delta^{13}C$ ($F_{1,28} = 8.49$, $P < 0.01$) and $\delta^{15}N$ ($F_{1,28} = 25.22$, $P < 0.01$). The species × season interaction was only significant for δD values ($F_{1,26} = 6.90$, $P = 0.02$). Multivariate and univariate analyses demonstrated that isotope values differed significantly between summer- and winter-grown feathers for all isotopes.

Northern Wheatears. Similar analyses showed no differences in δD values between adult summer-grown flight feathers and winter-grown back feathers in wheatears ($T_{0.05\,(2),\,14} = 0.74$, $P = 0.47$; Table 1.3). Nor were there differences in $\delta^{13}C$ ($T_{0.05\,(2),\,14} = 6.5$, P=0.72; Table 1.3) or $\delta^{15}N$ ($T_{0.05\,(2),\,14} = 25.5$, P = 0.12; Table 1.3) between flight and back feathers of northern wheatears.

Assignment Tests. Discriminant analyses were used to evaluate the ability to correctly classify plover feathers into their known group of origin based on the δD, $\delta^{13}C$, and $\delta^{15}N$ values of each feather. Pairwise analyses only considered two groups at any time in the assignment without an exclusion criterion. This method classified 82% of *P. fulva* and 80% of *P. dominica* winter- and summer-grown feathers into the correct groups of origin. However, this method correctly classified only 69% of the winter-grown feathers of the two species into their correct continental group, despite the differences in

Table 1.3. Stable isotope values (‰) obtained from summer- and winter-grown feathers of northern wheatears (*Oenanthe oenanthe*) collected in Alaska.

			$\delta D^{\dagger}$			$\delta^{13}C^{\dagger}$			$\delta^{15}N^{\dagger}$	
Age	Feather	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD	*n*	$\bar{x}$	SD
Adult	Summer	6	-132[a]	14	15	-21.38[b]	2.86	15	4.18[c]	2.62
Adult	Winter	10	-131[a]	28	16	-20.64[b]	3.47	16	5.24[c]	2.71

[†]Superscripts having at least one letter in common indicate no significant difference between samples, $P < 0.05$)

isotopic signatures from the feather's respective geographic origins. A 3-way comparison was used to classify summer-grown feathers of both plover species and the two interspecific groups of winter-grown feathers correctly assigned only 63% of the samples.

When an 80% probability of membership exclusion threshold was applied to posterior probabilities, only 41% of *P. fulva* winter- and summer-grown feathers and 30% of *P. dominica* feathers were assigned into correct groups of origin. No winter-grown feathers could be assigned to group of origin under the 80% exclusion threshold (none had a posterior probability of correct assignment higher than 75%). The 3-way comparison among the pooled summer-grown and the two groups of winter-grown feathers assigned 41% of feathers into correct groups at this exclusion threshold, a rate of success similar to random assignment.

Discussion

The inability to detect significant differences between isotope values in feathers grown on different continents is surprising, because δD, $\delta^{13}C$, and, to some extent $\delta^{15}N$, exhibit broad-scale geographic variation that should be reflected in feathers grown across such different latitudes and climates (Chamberlain et al. 1997; Hobson and Wassenaar 1997; Hobson 1999; Graves et al. 2002; Hobson 2002; Rubenstein et al. 2002). Although Alaska-grown northern wheatear feathers exhibited a strong geographic signature with little variation in δD (-140 ± 24 ‰; mean ± SD), Alaska-grown plover feathers did not have the consistent δD signature expected among feathers grown within a 100 km area. These feathers should have exhibited minimal variation (Hobson 1999) rather than broad

δD values of -84 ± 54 ‰ (mean ± SD). Based on isotopic maps of North America (Hobson 1999), isotopic ratios in these breeding-ground plover feathers are concordant with a breeding latitude between 55° to 45° N, rather than their true latitude of origin, approximately 65° N. These results cast doubt on the ability to assign wintering individuals to within better than several thousand km of their true breeding origins (see for example Hobson 1999), or to assess mixing of breeding populations on wintering areas using only isotopic markers.

Several factors, such as habitat and diet selection, element routing, and diet-tissue fractionation of stable isotopes (Gannes et al. 1997) may have caused much of the variation observed and compromised the ability to accurately assign feathers to their geographic origins. Variation in isotopic signatures among winter-grown feathers may be due to widespread geographic origins of growth, caused by either dispersed wintering among individuals or by an overlap of molt and migration. Interrupted or prolonged molt seems unlikely however, as *Pluvialis* plovers are swift migrants, and most individuals arrive on breeding and Alaska staging areas completely molted into alternate (dark) plumage (Johnson and Connors 1996). In addition, *P. fulva* that winter in Hawaii attain all their dark, alternate body feathers before migrating (O. Johnson, pers. comm.).

Intertidal marine feeding may be responsible for the variation in δD in summer- and winter-grown plover feathers and may explain some of the more positive δD values. However, the correlation between enriched deuterium and enriched $\delta^{13}C$ values that is expected from marine inputs was not exhibited in summer-grown feathers (Spearman's $\rho = 0.26$, $P = 0.352$). Moreover, only fresh feathers from birds collected on inland

Alaska breeding territories were used in these analyses. More positive deuterium values associated with snow melt, evaporation in drinking water sources, and relative humidity may play a role in some of the variation observed (Hobson et al. 1999; Wassenaar and Hobson 2000b), but controlled studies are necessary to assess the impact of these factors on isotopic ratios in feathers.

Feathers developed from nutrient stores from previously occupied habitats could also produce unexpected isotopic ratios. However, it is doubtful that nutrient stores from previous locations would persist after migration and throughout incubation and most of chick-rearing, due to the high energy costs associated with these activities (Gill 1990; Klaassen et al. 2001). Also, several studies have demonstrated that isotopic ratios in feathers reflect those in the diet at the time of growth (Mizutani et al. 1990; Hobson and Clark 1992). Furthermore, using $\delta^{13}C$, Klaassen et al. (2001) showed that 10 species of Arctic-breeding waders used local food sources for energetic requirements during the breeding season.

Assigning individuals to their populations or geographic origin based on their isotope ratios has a wide range of applications in management and conservation. However, confidence in an individual's assignment is critical to the usefulness of this method. I found that a liberal exclusion threshold of 80% dramatically decreased the acceptable number of correct assignments, demonstrating the weakness of these markers in these samples. In several of the analyses, feathers with a posterior probability of greater than 80% percent were misclassified. In a blind study attempting to assign feathers to unknown origin, these erroneous assignments would go undetected. Although

correlating isotopic signatures along latitudinal gradients is useful in some cases, the ability to track individuals or populations throughout their annual cycle based on isotopic ratios in their tissues depends on the development and implementation of an assignment method with robust exclusion criteria. At present, the assignment of individual feathers grown on widely separated continents with different climates and precipitation regimes is no better than random.

Even isotopic markers with pre-migratory (breeding ground) signatures were inadequate to delineate high latitude breeding areas, suggesting that tracking migrants will require more than just a knowledge of isotope gradients in the range of the study organism (Hobson et al. 2001; Hobson 2002). These results also demonstrate that isotopic gradients on continents other than North America may not follow predictable patterns or are too complex to distinguish feathers grown on breeding grounds from those grown on wintering areas. These results across different species and different migration systems deflate some of the promise of these markers as a universal tool for linking highly mobile animals to geographic areas of biological importance to them.

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CHAPTER TWO

BIOMONITORING OF CONTAMINANTS AMONG BIRDS FROM TWO TROPHIC LEVELS IN THE NORTH PACIFIC

Abstract

The presence and accumulation of persistent contaminants at high latitudes from long-range transport is an important environmental issue. Atmospheric transport has been identified as the source of pollutants in several arctic ecosystems and has the potential to severely impact high-latitude populations. Elevated levels of contaminants in Aleutian Island avifauna have been documented on several islands, but the great distance from potential industrial sources and the region's complex military history has confounded identification of contaminant origins. Identifying sources of pollution and documenting ecosystem concentrations is necessary to implement mitigation for point sources and gain support for reducing global emissions of atmospherically transported compounds. I sampled bird species (cormorants and sandpipers) across the natural longitudinal transect of the Aleutian Archipelago to test three sources for contaminants: (1) long-range transport, (2) point sources, and (3) migratory prey. Stable isotopes were used to confirm trophic and non-migratory status in cormorants. Carbon isotopic ratios in cormorants were distinct among islands, enabling us to link cormorants to local island food webs and rule out transfer through migratory prey as a contaminant source. I detected patterns in some PCB congeners and mercury that indicate abandoned military installations as likely local point sources. The long-range transport hypothesis was supported by significant

west-to-east declines in contaminant concentrations for the majority of detected organochlorines and trace metals. Although relatively low at present, concentrations are likely to increase in Aleutian fauna as Asian industrialization increases and emitted contaminants are atmospherically transported into the region, necessitating continued monitoring in this unique ecosystem.

Introduction

The North Pacific Ocean and Bering Sea host large numbers of seabirds and some of the world's most productive fisheries, making them important regions of high-latitude biodiversity and productivity. The Aleutian Archipelago defines the border between these two water bodies and provides nesting sites for some of the largest seabird colonies in North America and to many endemic bird populations (e.g., Murie 1959). Although remote and largely uninhabited by humans, the Aleutian Islands have been impacted by many anthropogenic activities, such as the harvest of natural resources (Merrick 1997), introduction of exotic species (Bailey 1993), military activities (Garfield 1969), and most recently, contaminants (Estes et al. 1997; Anthony et al. 1999). Aleutian birds are especially vulnerable to anthropogenic influences due to their restricted ranges, and, in the case of some endemics, small population sizes (Murie 1959). Thus, these birds can also serve as important biomonitors in this biologically and economically important region.

Elevated concentrations of contaminants can cause both acute and chronic health effects. Chronic effects may include impairment to reproduction, behavior, neurological function, and suppressed immune function (Walker et al. 1996). The most toxic

organochlorines (OCs) are lipophilic compounds that are stored and accumulate in fatty tissues (Hoffman et al. 1996). Year-round residents in arctic and sub-arctic environments are dependant on fat reserves as a buffer against cold, stress, and periods of low food availability (Arctic Monitoring and Assessment Programme [AMAP] 1998), a characteristic that may contribute to elevated contaminant concentrations in these organisms. Metabolism of fat stores may have a negative impact by releasing stored OCs into the blood or increasing the concentration in remaining fat to toxic levels (Walker et al. 1996). Thus, the storage and metabolic dependence upon fat may make many non-migratory Aleutian birds especially vulnerable, even when contaminants are present at relatively low levels in the environment.

Organic and trace metal contaminants have been implicated in low productivity in non-migratory bald eagles (*Haliaeetus leucocephalus*) on several Aleutian Islands (Estes et al. 1997; Anthony et al. 1999; Stout and Trust 2002). Although the origin of contamination in the Aleutian Archipelago remains undetermined, three hypotheses exist regarding contaminant origins in this region: point sources, global transport, and migratory prey. Past military sites have produced locally high concentrations of polychlorinated biphenyls (PCBs) due to the improper disposal of electrical equipment (AMAP 1998) and are considered a potential source for continued contamination in the Aleutian Islands (Anthony et al. 1999; Stout and Trust 2002). Bald eagle eggs from islands that were occupied by military installations had the highest PCB concentrations, a factor used to advance the hypothesis that historic military activities are point sources of Aleutian Island pollution (Estes et al. 1997; Anthony et al. 1999).

Atmospheric and oceanic pathways play large and potentially different roles in the global distribution of contaminants and have been implicated as a major source of persistent organic pollutants in Pacific and arctic ecosystems (Barrie et al. 1992; Iwata et al. 1993, 1994; Simonich and Hites 1995; Wikening et al. 2000; Borga et al. 2002). Contaminants are transported in the atmosphere from sources in warm regions to colder climates, where they condense and precipitate into the ecosystem (Wanis and Mackay 1993; Simonich and Hites 1995). The Aleutian Low pressure that dominates weather over the North Pacific and Bering Sea during winter months draws both storms and airborne contaminants from southeast Asia eastward along the Aleutian Archipelago (Stabeno et al. 1999; AMAP 2002). A west-to-east decrease in contaminant concentrations along the Aleutian Archipelago is expected in atmospherically transported contaminants due to the prevailing weather patterns and the fact that atmospheric concentrations tend to decrease with distance from the source (Iwata et al. 1994). This hypothesis was forwarded to explain the high organochlorine and DDE concentrations found in bald eagle eggs from western Aleutian Islands (Estes et al. 1997; Anthony et al. 1999).

Pollutants may also be introduced into the Aleutian food web through migratory piscine and avian prey species. Many Aleutian breeding birds winter in coastal areas in Washington, Oregon, and California (American Ornithologists' Union 1997) where contaminants have been documented in marine food webs (Brown et al. 1998), and may be transferred to predatory birds that prey upon them (Anthony et al. 1999).

The Aleutian Islands provide a natural experimental longitudinal transect across the North Pacific to test hypotheses of contaminant sources. The objective of this study was to quantify organic and trace metal contaminant concentrations in birds from two Aleutian food webs to test the three contaminant source hypotheses forwarded by Anthony et al. (1999). I used contaminant concentrations in non-migratory birds from two food webs to assess the impact of point source and long-range transport at five islands along the Aleutian Archipelago. Stable isotopes were used to examine the possible confounding factor of migratory prey species in the top trophic feeders studied. Isotopic signatures of primary producers and plankton are transferred up the pelagic food chain (Hobson and Montevecchi 1991; Hobson and Welch 1992; Hobson 1993; Hobson et al. 1994) in a predictable, step-wise manner (DeNiro and Epstein 1981) and these isotopes can be used to delineate local food webs and infer foraging locations avian predators (Hobson and Welch 1992; Hobson 1993). Therefore, I hypothesized that the regionally distinct carbon isotope values in Aleutian plankton (Saupe et al. 1989; Schell et al. 1998) would be detected in birds feeding in local, non-migratory food webs.

Materials and Methods

Focal Species. Pelagic and red-faced cormorants (*Phalacrocorax pelagicus* and *P. urile*) are year-round, non-migratory residents in the Aleutian Islands, where they often co-occur in breeding colonies and foraging areas (Hobson 1997; Causey 2002). Both species are exclusively marine, but they prefer inshore and coastal habitats where they feed predominately on benthic, solitary fish (Hobson 1997; Causey 2002). Both cormorant species were used to assess contaminant loads in top predators of coastal

Aleutian Island food webs. Collection efforts focused at the generic level (*Phalacrocorax* spp.) due to the ecological similarity of the two species. Subsequent statistical analyses detected no differences in contaminant concentrations between the two species, confirming the decision to pool the two as ecologically similar (see below).

Aleutian rock sandpipers (*Calidris ptilocnemis*) are also non-migratory residents in the Aleutian Islands (Gill 2003), and were used to assess contaminant concentrations at a lower trophic level. Rock sandpipers feed on inshore benthic invertebrates, except during breeding when birds forage predominantly on their tundra territories for terrestrial invertebrates (Gill 2003). Specimens were collected early in the breeding season before nest initiation only from inshore habitats.

Study Area. The Aleutian Archipelago includes over 200 islands, extends more than 3,500 km from the Alaska mainland, and defines the border between the North Pacific Ocean and the Bering Sea. The study area consisted of five islands in this archipelago, representing a west-to-east geographical gradient (Attu, Kiska, Adak, Amlia, and Amak/Alaska Peninsula; Fig. 2.1). Logistical constraints precluded the collection of rock sandpipers on Kiska Island.

Sample Collection and Preparation. Pelagic and red-faced cormorants ($n = 76$) and rock sandpipers ($n = 38$) were collected in 2000 and 2001. Each specimen was weighed, tagged, and frozen until preparation. In the laboratory, birds were thawed and morphological measurements (wing chord, tarsus, tail, bill, and skull lengths, bill width, and bill height) taken to the nearest 0.1 mm. After harvesting tissue samples for chemical and isotopic analyses (described below) all specimens were preserved and

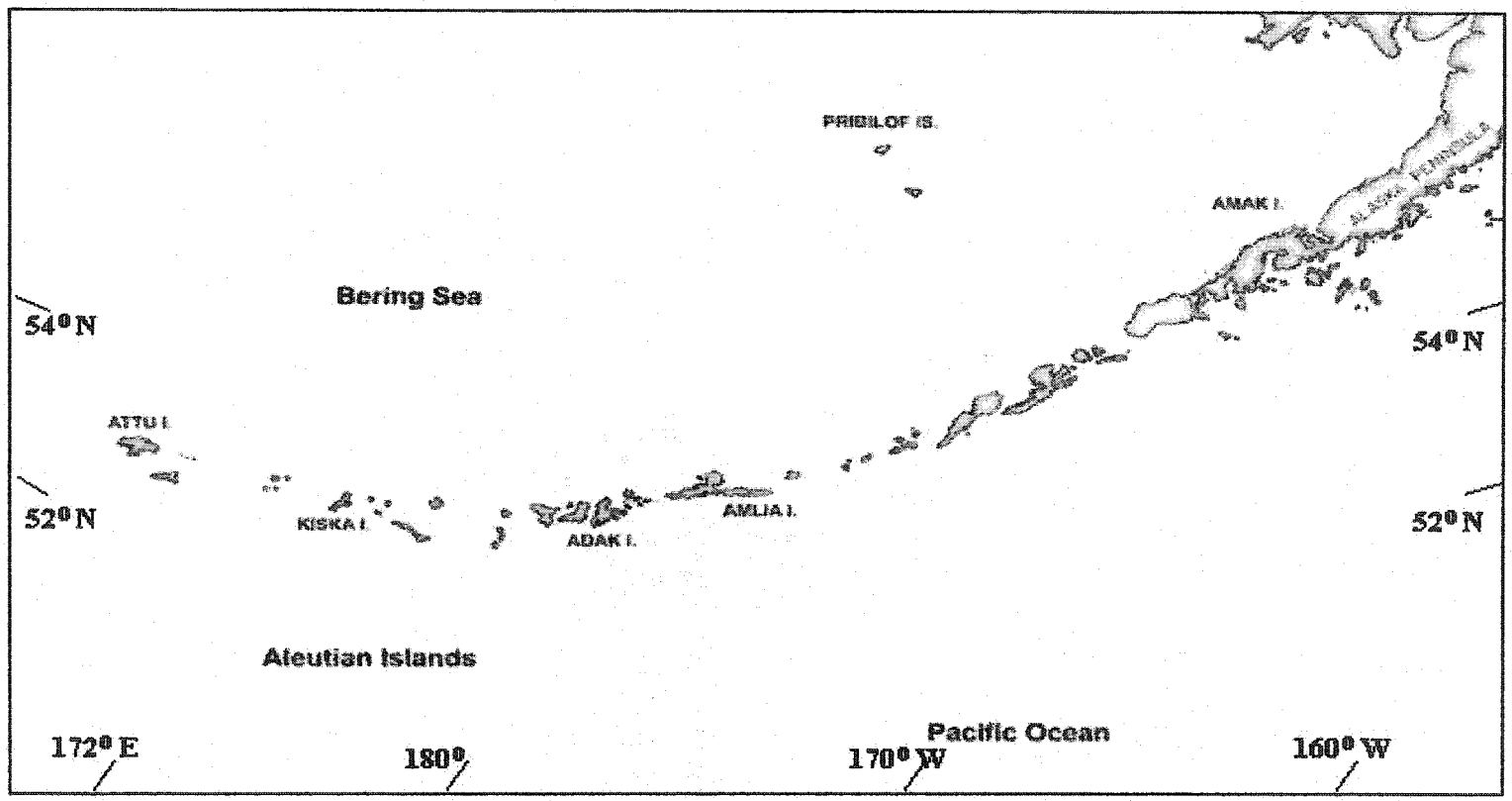

Figure 2.1. Sampling sites in the Aleutian Archipelago, Alaska.

archived at the University of Alaska Museum as skins, skeletons, and tissue and stomach samples (after Winker 2000).

Contaminant Analyses. Liver and breast muscle samples were removed from specimens using chemically clean instruments (hexane-acetone wash) and new, sterile, stainless steel scalpel blades were replaced after each sample was removed. Due to the small body size of rock sandpipers, liver and muscle tissues from 2 individuals from the same site were randomly combined to obtain adequate sample mass of each tissue for analyses. Samples were placed in separate, chemically clean, glass jars (I-CHEM) and frozen at –20° C until shipped to the analytical laboratory. Environmental Research Institute (Storrs, CT) performed the elemental and organochlorine analyses.

Elemental Analyses. Samples were analyzed for nine different metals. Arsenic, cadmium, chromium, copper, nickel, lead, selenium, and zinc were analyzed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a modified NOAA ORCA 130 Methods (Qian et al. 1997a). The method detection limits (MDLs) for these techniques vary by target analyte, but ranged from 0.03 to 0.5 mg/Kg. Tissues were analyzed for total mercury by Cold Vapor Atomic Absorption Spectroscopy using EPA Method 245.6. The MDL for this method was 0.2 μg/Kg. Percent moisture was calculated as the percent difference between the wet (initial) weight and the dry (final) weight of a homogenized sub-sample that was dried for at least 16 hours at 105 ° C.

Organochlorine Analyses. Samples were analyzed for 28 chlorinated organic compounds (aldrin; alpha, beta, delta, and gamma benzene hexachloride [BHC]; alpha and gamma chlordane; cis and trans nonachlor; dieldrin; endosulfan I and II; endosulfan-

sulfate; endrin, endrin aldehyde and ketone; heptachlor; heptachlor-epoxide; hexachlorobenzene [HCB]; methoxychlor; mirex; oxyochlordane; o,p'-DDD; o,p'-DDE; o,p'-DDT;, p,p'-DDD; p,p'-DDE; p,p'-DDT) and 20 PCB congeners (8, 18, 28, 44, 52, 66, 77, 101, 105, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, and 209). Tissues were extracted for organic target compounds by automated solvent extraction (ASE 2000-Dionex Corp. Sunnyvale, CA) using methylene chloride. Extracts were analyzed for pesticides and PCB congeners using a modified NOAA ORCA 130 Method (Qian et al. 1997b). A gas chromatograph (GC) was equipped with a dual micro-electron capture detector, a liquid auto sampler, and two capillary columns. The MDLs for this method varied by target compound, but ranged between 0.00025 and 0.0025 mg/Kg. Percent lipid was determined by drying two weighed subsamples at 105 ° C for 30 minutes and re-weighing. The percent lipid was calculated as the percent lipid of wet sample weight.

Analytical accuracy was assessed using duplicate analyses for 10% of the tissue samples. Spiked sample recovery and procedural blanks were used for 5% of the samples. Data were considered acceptable if the spiked recoveries were □ 85% and □ 115% of expected values and the relative percent difference between a sample and its corresponding duplicate was □ 20%.

Isotopic Analysis. Breast muscle samples were placed in separate, clean, plastic tubes (cryotubes) and frozen at –20° C until analysis. Prior to analyses, tissues were dried at 60-70° C for 48 h and then ground to a fine powder with a mortar and pestle. Samples were weighed (1 - 1.5 mg) into tin cups and analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the mass-spectrometry facility, University of Alaska Fairbanks, using a Europa 20/20 continuous

flow isotope ratio mass-spectrometer. Samples were analyzed in duplicate and results were accepted if the variance between the duplicates was not greater than the variance of the peptone standard (Rosing et al. 1998). Isotopic ratios are reported as per mil (‰) deviation from the standard as defined by:

$$\delta X=([R_{sample} / R_{standard}] - 1) \times 1000,$$

where X is ^{13}C or ^{15}N, and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standards were PeeDee Belemnite (C) and atmospheric N_2 (N).

Data Analyses. Organochlorine (OC) concentrations in breast muscle and trace metal concentrations in livers were compared within each genus among the five sites for cormorants and among the four sites for rock sandpipers. Analytes with > 50% of all samples below the limit of detection (LOD) were not statistically analyzed. Non-detect values of statistically analyzed compounds were substituted with random numbers between zero and the LOD for each sample (Helsel 1990). In addition to site differences, species (cormorants), sex (cormorants only; sandpiper samples were pooled randomly by sex), year, and interaction terms were included in initial models. Terms were included in the final model only if univariate *P* values were < 0.05. Data were predominantly non-normal ($P < 0.05$, Shapiro Wilk), but homoscedastic ($P > 0.05$, Bartlett's Test), and because linear models are robust under these conditions (Sokal and Rohlf 1995), I used parametric statistical tests for all analyses. All statistical analyses were performed in the Statistical Analysis System (1999, version 8e; Cary, NC).

Identifying possible point sources required testing for differences among sites. For these analyses, I used a multivariate ANCOVA (analysis of covariance) design with

site as a factor, percent lipid as a covariate, and contaminant concentrations as the response variables for OCs. I used a multivariate ANOVA (analysis of variance) with site as the main factor and contaminant concentrations as the response variables for trace metals. If an analyte had > 50% of samples below the LOD at one site, that site was excluded from the analysis (Helsel 1990). If the overall multivariate model showed significant differences among sites, univariate analyses were performed on analytes with significant ($P < 0.05$) univariate F-statistics and were Bonferroni-adjusted for multiple comparisons among sites. Correlation analyses (Spearman's ρ) were used to detect any association among morphological characters and contaminants and were Bonferroni-adjusted based on the number of contaminants in the analyses.

Patterns suggesting atmospheric transport (i.e., west-to-east decline in contaminant concentrations) could only be tested through regression. I used linear regression models to investigate relationships between relative longitude (longitudinal differences among sites with Attu set artificially to 0^{o}) and concentrations of atmospherically transported contaminants and stable isotope values. To correctly test this hypothesis, regressions were conducted with all sites (including sites with > 50% non-detects) included in the analyses under the presumption that low rates of detection were correlated with low concentrations. For these analyses, all non-detects were substituted with random numbers between zero and the LOD for each sample (Helsel 1990). All critical *P*-values were adjusted for multiple comparisons among sites using the Bonferroni correction procedure (Sokal and Rohlf 1995).

Results

Organochlorines (OCs)

Cormorants. Six compounds were detected in sufficient quantities to permit statistical analyses in cormorants: HCB, p,p'-DDE, PCB-138, PCB-153, ΣPCBs (sum of all quantified congeners), and trans-nonachlor (Table 2.1). Although uncommon among all sites (i.e., occurring in fewer than 50% of total samples), dieldrin was detected in > 50% of the samples at Kiska and Adak, and PCB-180 was detected in > 50% of samples from Attu and Adak (Table 2.1). Other organochlorines previously detected in the region (Estes et al. 1997; Anthony et al. 1999) were rare (mirex) or not detected (p,p'-DDD).

Multivariate analysis indicated significant differences in contaminant levels in cormorants among sites ($F_{24,228} = 2.98$, $P < 0.01$; Wilk's lambda). There were no significant differences between species, sexes, or for the year × site interaction effect. Year was only marginally significant ($F_{6,45} = 2.37$, $P = 0.05$) and therefore not included in the subsequent univariate analyses.

PCB congeners 138, 153, and ΣPCBs were the only OCs that differed significantly among sites ($F_{5,70} = 9.73$, $P < 0.01$; $F_{5,70} = 7.45$, $P < 0.01$; $F_{5,70} = 8.24$, $P < 0.01$; respectively). All the PCBs had the same pattern with the highest concentrations in birds from Adak and Attu (Table 2.1). All detected OCs were positively correlated with each other ($P < 0.01$, Spearman's ρ); however, correlation analysis failed to detected any association between OC concentrations in muscle tissue and morphological characters or mass ($P > 0.05$, Spearman's ρ). Regressions of OCs on relative longitude were

Table 2.1. Geometric means (and ranges) for organochlorine concentrations (ppm wet wt.) in muscle tissue of cormorants (*Phalacrocorax* spp.) collected in 2000 and 2001from the Aleutian Islands, Alaska.

Analyte[1]	Attu (n = 23)	Kiska (n = 12)	Adak (n = 12)	Amlia (n = 19)	Amak (n = 10)
dieldrin	NA[2]	0.0012	0.0022	NA	ND
		(ND – 0.003)	(ND – 0.005)		
HCB	0.0033	0.0023	0.0026	0.0026	0.0011
	(ND – 0.029)	(ND – 0.005)	(0.001 – 0.006)	(ND – 0.005)	(ND – 0.001)
p,p'-DDE	0.0095	0.0029	0.0136	0.0090	0.0017
	(ND – 0.088)	(ND – 0.013)	(0.009 – 0.024)	(ND – 0.028)	(ND – 0.003)
PCB 138	0.0065[ab]	0.0015[b]	0.0172[a]	0.0023[b]	ND[3]
	(ND – 0.057)	(ND – 0.006)	(0.005 – 0.036)	(ND – 0.006)	
PCB 153	0.0085[ab]	0.0015[b]	0.0307[a]	0.0059[b]	ND
	(ND – 0.106)	(ND – 0.004)	(0.011 – 0.097)	(ND – 0.018)	

Table 2.1 continued.

Analyte[1]	Attu (n = 23)	Kiska (n = 12)	Adak (n = 12)	Amlia (n = 19)	Amak (n = 10)
PCB 180	0.0063	NA	0.0149	NA	ND
	(ND – 0.036)		(ND – 0.005)		
total PCB	0.0260^{ab}	0.0023^{b}	0.0716^{a}	0.0055^{b}	ND
	(ND – 0.275)	(ND – 0.010)	(0.025 – 0.233)	(ND – 0.033)	
trans-	0.0048	0.0010	0.0035	0.0029	0.0012
nonachlor	(ND – 0.038)	(ND – 0.001)	(0.003 – 0.005)	(ND – 0.011)	(ND – 0.002)

[1] Analytes having different letter superscripts indicate significant differences at the $P < 0.01$ level,

[2] NA = not calculated because analyte was detected in < 50% of samples.

[3] ND = not detected in any samples (LOD < 0.0006 ppm)

significant ($P < 0.05$) for four contaminants (HCB, DDE, trans-nonalchlor, and PCBs) and in all cases, concentrations decreased from west to east (Fig. 2.2).

Rock Sandpipers. Only p,p'-DDE was detected in enough rock sandpipers to permit an ANCOVA test. DDE differed among sites ($F_{4,14} = 16.33$, $P < 0.01$; ANCOVA) with Adak birds having the highest concentrations among islands (Table 2.2). No linear relationship was detected between p,p'-DDE and relative longitude ($F_{1,17} = 0.02$, $R^2 < 0.01$, $P = 0.89$). PCB congeners 138 and 153 were detected in all Adak and most (67%) Attu samples (Table 2.2).

Metals

Cormorants. Arsenic, cadmium, chromium, selenium, zinc, and mercury were detected in enough samples to permit comparisons. Multivariate analysis of these metals indicated significant differences among sites ($F_{20,94} = 3.55$, $P < 0.01$). Year, sex, and the interaction terms were not significant ($P > 0.05$).

Overall, elemental metal concentrations were highly variable (Table 2.3). Univariate ANOVAs detected differences in cadmium ($F_{4,70} = 6.50$, $P < 0.01$), mercury ($F_{4,72} = 6.50$, $P = 0.02$), and selenium ($F_{4,44} = 3.75$, $P = 0.01$) among sites. Multiple pairwise comparisons indicated that cadmium concentrations were highest on Attu and significantly lower on Amak (Table 2.3). Mercury concentrations were extremely variable (Table 2.3), and post-hoc tests did not identify the source of variation. Although selenium concentrations were highest on Attu, they differed significantly only from Kiska (Table 2.3). Linear regressions detected significant relationships between arsenic and cadmium and the independent variable, relative longitude (Fig. 2.3), but a linear

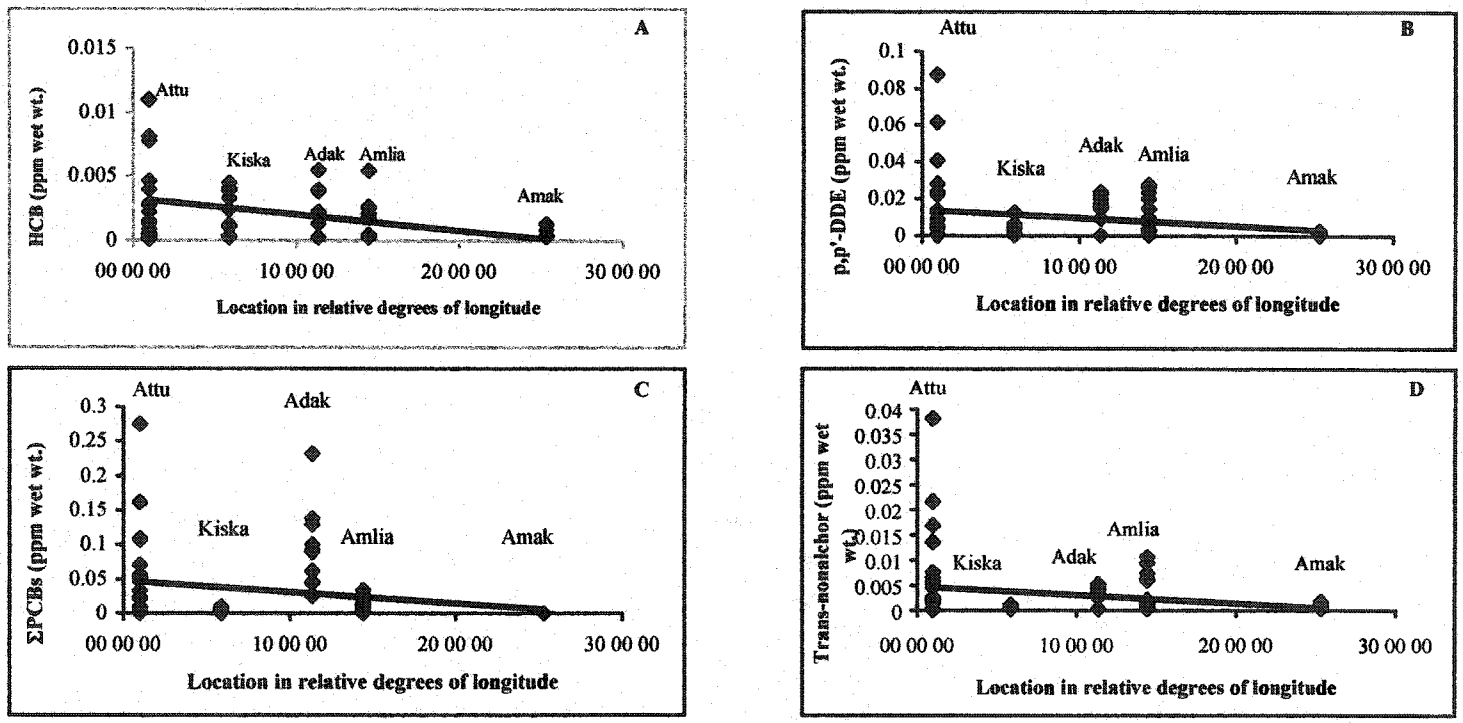

Figure 2.2. Relationships between atmospherically transported organochlorines in muscle tissue (see Table 2.1 for sample sizes) of cormorants (*Phalacrocorax* spp.) and relative longitude of sampled Aleutian Islands, Alaska. A) HCB (HCB = -1.24 x 10^{-8}[longitude] + 0.03, $R^2 = 0.07$, $P = 0.02$); B) p,p'-DDE (DDE = -4.21 x 10^{-8} [longitude] + 0.01, $R^2 = 0.06$, $P = 0.04$); C) ΣPCB (ΣPCB = -1.62 x 10^{-7}[longitude] + 0.05, $R^2 = 0.06$, $P = 0.03$); and D) trans-nonachlor (trans-nonachlor = -1.67 x 10^{-8}[longitude] + 0.01, $R^2 = 0.07$, $P = 0.04$).

Table 2.2. Geometric means (and ranges) for elemental concentrations (ppm dry wt.) in livers and p,p'-DDE concentrations and total PCBs (ppm wet wt.) in muscle of rock sandpipers (*Calidris ptilocnemis*) collected in 2000 and 2001 from the Aleutian Islands, Alaska.

Metal[1]	Attu ($n = 6$)[2]	Adak ($n = 6$)	Amlia ($n = 4$)	AK Pen ($n = 3$)
arsenic	6.58[a]	2.09[b]	1.34[b]	3.52[ab]
	(4.01 – 8.17)	(1.36 – 3.27)	(1.04 – 1.69)	(2.01 – 8.12)
cadmium	7.61	5.96	6.88	4.65
	(5.03 – 10.02)	(4.72 – 7.36)	(5.18 – 9.55)	(3.66 – 5.65)
chromium	5.68	5.83	6.15	6.56
	(ND[3] – 7.77)	(5.26 – 6.54)	(5.65 – 7.05)	(ND – 6.50)
mercury	0.54[a]	1.12[a]	0.92[a]	8.48[b]
	(0.08 – 1.02)	(0.14 – 3.87)	(0.31 – 2.29)	(4.13 – 13.95)
nickel	1.03	0.09	0.11	0.08
	(ND – 3.57)	(0.05 – 0.17)	(0.07 – 0.17)	(ND – 0.10)
selenium	14.12	11.01	15.82	24.27
	(5.25 – 24.59)	(6.54 – 22.35)	(5.56 – 35.36)	(12.16 – 60.94)

Table 2.2. continued.

Metal[1]	Attu ($n = 6$)[2]	Adak ($n = 6$)	Amlia ($n = 4$)	AK Pen ($n = 3$)
p,p'-DDE	0.0015[a]	0.0031[b]	0.0008[a]	0.0012[a]
	(ND – 0.0089)	(0.0017 – 0.0045)	(ND – 0.0014)	(ND – 0.0015)
ΣPCB[3]	0.0031	0.0045	ND[4]	ND
	(ND – 0.0017)	(0.0020 – 0.0120)		

[1] Analytes having different letter superscripts indicate significant differences at the $P < 0.01$ level.

[2] Sample sizes = 2 individuals combined for each value of n (see methods).

[3] Sum of PCB congeners 138 and 153

[4] ND = not detected (LOD < 0.0006 ppm)

Table 2.3. Geometric means (and ranges) for elemental concentrations (ppm dry wt.) in cormorant (*Phalacrocorax* spp.) livers collected in 2000 and 2001 from the Aleutian Islands, Alaska.

Metal[1]	Attu (n = 23)	Kiska (n = 12)	Adak (n = 12)	Amlia (n = 20)	Amak (n = 10)
arsenic	1.18	0.74	1.13	1.14	1.58
	(0.27 – 4.02)	(0.40 – 1.16)	(0.77 – 1.67)	(0.38 – 2.92)	(1.08 – 2.96)
cadmium	3.04[a]	1.21[b]	2.05[ab]	1.92[ab]	0.85[b]
	(ND – 12.93)	(0.39 – 2.81)	(0.83 – 4.56)	(0.86 – 6.61)	(0.33 – 2.01)
chromium	4.98	3.77	4.07	4.40	5.40
	(2.24 – 17.69)	(3.18 – 5.16)	(3.17 – 7.50)	(2.70 – 9.36)	(4.41 – 6.72)
copper	20.30	20.53	17.33	9.27	ND[2]
	(14.86 – 45.14)	(14.14 – 28.76)	(7.07 – 29.08)	(0.60 – 20.59)	

Table 2.3 continued.

Metal	Attu	Kiska	Adak	Amlia	Amak
mercury	3.69	1.45	2.11	3.45	3.89
	(0.16 – 27.66)	(0.49 – 5.63)	(0.89 – 5.03)	(0.34 – 14.15)	(1.45 – 7.51)
selenium	16.95[a]	5.70[b]	13.67[ab]	12.86[ab]	19.33[a]
	(4.25 – 39.64)	(5.01 – 6.32)	(9.65 – 26.17)	(7.31 – 24.82)	(11.63 – 39.07)
zinc	86.04	88.82	68.79	27.67	NA[3]
	(63.19 – 273.26)	(69.92 – 117.36)	(36.13 – 84.37)	(0.60 – 79.33)	

[1]Elements having different letter superscripts indicate significant differences at the $P < 0.01$ level.

[2] ND = not detected (LOD < 0.59 ppm).

[3] NA = not analyzed for this location.

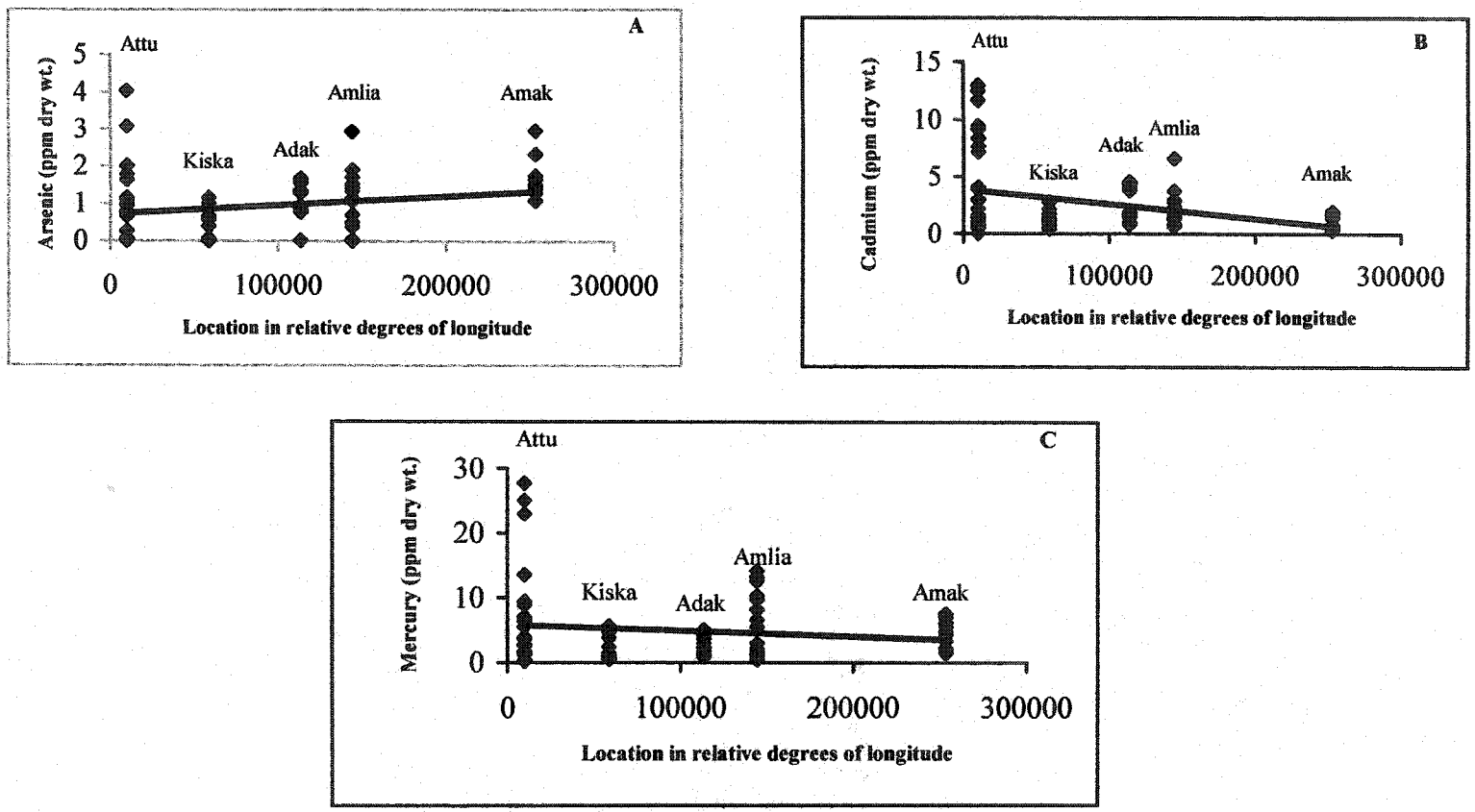

Figure 2.3. Relationships between atmospherically transported trace elements in liver tissue (see Table 2.2 for sample sizes) of cormorants (*Phalacrocorax* spp.) and relative longitude of sampled Aleutian Islands, Alaska. A) Arsenic (As = -2.4 x 10^{-6}[longitude] + 0.72, $R^2 = 0.05$, $P = 0.05$); B) cadmium (Cd = -1.28 x 10^{-5}[longitude] + 3.89, $R^2 = 0.13$, $P < 0.01$); and C) mercury (Hg = -8.9 x 10^{-6}[longitude] + 5.82, $R^2 = 0.02$, $P = 0.26$).

relationship was not detected for mercury (Fig. 2.3) or selenium. Mercury was positively correlated with selenium ($r = 0.4975$, $P < 0.01$; Spearman's ρ).

Rock Sandpipers. Seven metals (arsenic, cadmium, chromium, nickel, lead, mercury, lead, and selenium) were detected in sufficient quantities to permit statistical analyses (Table 2.2). Multivariate analysis showed a significant difference among sites ($F_{3,21} = 4.98$, $P = 0.02$), and subsequent univariate analyses detected significant differences among sites in arsenic ($F_{3,15} = 8.09$, $P < 0.01$) and mercury concentrations ($F_{3,15} = 13.00$, $P < 0.01$; Table 2.2). Concentrations of arsenic and cadmium decreased significantly with relative longitude, but significantly increased in mercury (Fig. 2.4). Unlike the cormorants, mercury was not correlated with selenium in sandpipers (Spearman's $\rho = 0.04$, $P = 0.90$).

Stable isotopes. Lipids tend to show depleted levels of $\delta^{13}C$ relative to other tissues (Gannes et al. 1997; Thompson et al. 2000), and therefore isotope ratios in tissues may be affected by lipid content. Multivariate analysis of $\delta^{13}C$, $\delta^{15}N$, and lipid (MANCOVA) detected significant differences among sites ($F_{5,42} = 34.63$, $P < 0.01$). Univariate tests detected differences among sites in both nitrogen and carbon ($F_{4,42} = 5.26$, $P < 0.01$ and $F_{4,42} = 25.64$, $P < 0.01$, respectively). Although nitrogen isotope values in muscle tissue had a small range among islands (range 10.36 – 11.64‰), multiple pairwise comparisons detected significant difference between the highest $\delta^{15}N$ value from Adak and the lowest values from Kiska ($P < 0.01$). Location, lipid, and the interaction (lipid x location) had significant effects on $\delta^{13}C$ ratios ($F_{4,39} = 8.89$, $P < 0.01$, $F_{1,39} = 5.22$, $P = 0.03$, and $F_{4,39} = 4.28$, $P < 0.01$, respectively). Carbon values were

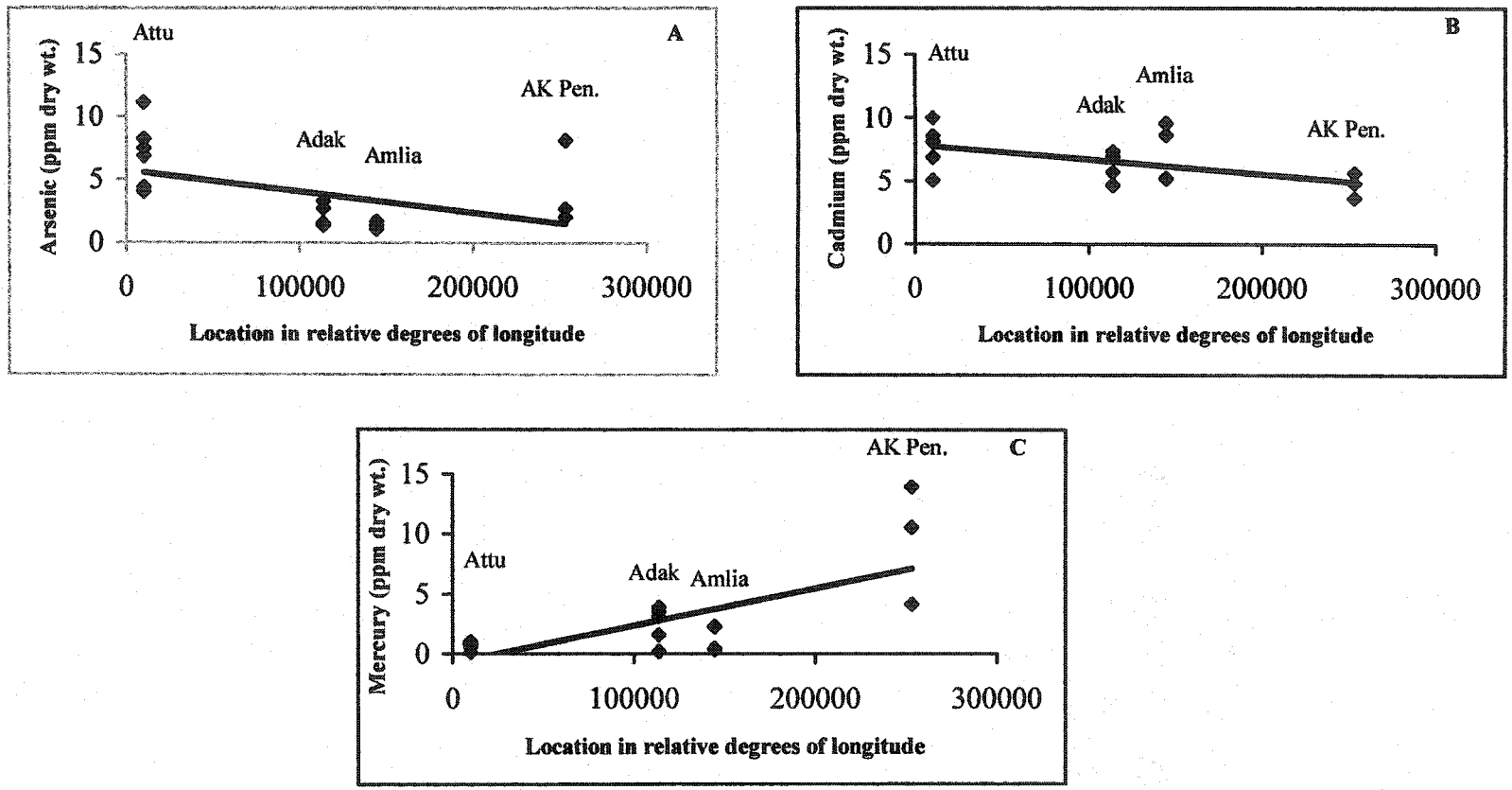

Figure 2.4. Relationships between atmospherically transported trace elements in liver tissue (see Table 2.3 for sample sizes) of rock sandpipers (*Calidris ptilocnemis*) and relative longitude of sampled Aleutian Islands, Alaska. A) Arsenic (longitude = -1.65×10^{-5}[longitude] + 5.69, $R^2 = 0.21$, $P = 0.05$); B) cadmium (longitude = -1.15×10^{-5}[longitude] + 7.88, $R^2 = 0.27$, $P = 0.02$); and C) mercury (Hg = -3.13×10^{-5}[longitude] + 0.77, $R^2 = 0.51$, $P < 0.01$).

significantly lower on Attu and Kiska than on Adak, Amlia, and Amak ($P < 0.01$; Fig. 2.5). Regression analysis detected a significant increase in $\delta^{13}C$ values in cormorant muscle tissue with relative longitude ($F_{1,47} = 78.89$, $P < 0.01$, $R^2 = 0.63$; Fig. 2.5). No such relationship existed for $\delta^{15}N$ and longitude ($F_{1,47} < 0.00$, $R^2 < 0.01$, $P = 0.96$). Arsenic and PCBs were the only analytes correlated with $\delta^{15}N$ or $\delta^{13}C$ ($P < 0.05$, Spearman's ρ), suggesting a slight biomagnification effect in cormorants for these compounds.

Discussion

These results reveal a strong pattern of west-to-east declines among contaminants in cormorants and rock sandpipers in the Aleutian Islands. Atmospheric transport is the only hypothesis proposed that explains these overarching results. This pattern indicates that subarctic regions are susceptible to the same atmospheric deposition that occurs in some regions of the arctic (Muir et al. 1992; Barrie et al. 1992; Iwata et al. 1993 and 1994; Simonich and Hites 1995; Bard 1999; Wikening et al. 2000). Linear regressions were used only to test for longitudinal relationships with contaminant concentrations, not to serve as predictive models. *P* values for some analytes were above the adjusted alpha levels for multiple comparisons, but the overwhelming percentage of significant relationships belies the possibility that these positive findings are a result of increased experiment-wise error. Despite the high variability in contaminant concentrations among individuals (i.e., low R^2 values), which is common in mobile organisms, these data consistently revealed patterns concordant with the atmospheric transport hypothesis.

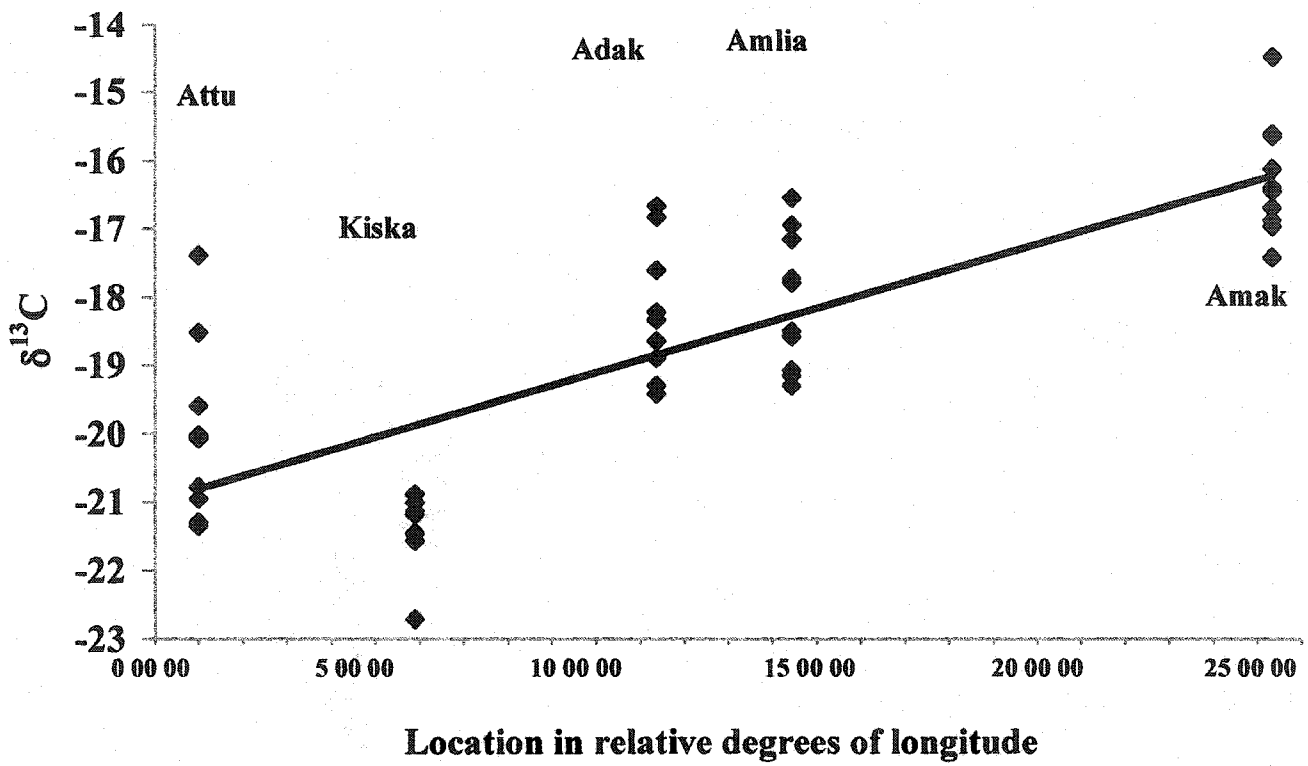

Figure 2.5. Relationship between carbon isotope ratios (‰) in muscle tissue (n = 10 for all sites) of cormorants (*Phalacrocorax* spp.) and relative longitude of sampled Aleutian Islands, Alaska. $\delta^{13}C = -20.99 + 0.01$(longitude), $R^2 = 0.63$, $P < 0.001$.

Although many of the PCBs found at high latitudes are globally transported (AMAP 1998), past military installations are considered a major point source in the Aleutian Islands (White and Risebrough 1977; Stout and Trust 1997; Anthony et al. 1999; Bacon et al. 1999; Crayton 2000; Stout and Trust 2002). These data suggest that both sources may be responsible for PCB distribution in the Aleutian Islands. The highest mean concentrations of PCBs in cormorants (Table 2.1), and the only detected PCBs in rock sandpipers (Table 2.2), were from Attu and Adak. Although the military histories of these islands are very different, these patterns suggest that islands contribute point source PCBs at two trophic levels.

Significant differences in cadmium and selenium among sites in cormorants (Table 2.3) had no discernible pattern and therefore may reflect unknown point sources. Mercury concentrations increased significantly from west-to-east in rock sandpipers, a pattern that is inverse to the expected long-range atmospheric transport distributional pattern. High mercury concentrations in rock sandpipers from the Alaska Peninsula drive this relationship (Fig. 2.4c) and this may indicate an eastern point source from military operations (Garfield 1969) or mercury deposits in the region (AMAP 2002).

I found no evidence to suggest that contaminants were transported into local food webs by migratory prey species. The eastern enrichment of $\delta^{13}C$ values was significant in cormorant muscle (Fig. 2.5) and reflects the trend of west-to-east enrichment of $\delta^{13}C$ found in plankton along the Aleutian Archipelago (Saupe et al. 1989; Schell et al. 1998). Although no direct comparison could be made between patterns in this study and patterns found by Schell et al. (1998), the similar trend of $\delta^{13}C$ values between plankton and

cormorants implies that, during this study, cormorant populations were feeding in non-migratory, localized food webs.

For PCBs, congeners 138 and 153 were frequently detected in this study. These two congeners are prevalent in aquatic biota (Eisler and Belisle 1996) and are routinely detected in marine and arctic food webs (AMAP 1998; Bacon et al. 1999). Toxicity thresholds for PCBs are congener-specific, and toxic equivalency factors (TEF) enable direct comparisons among studies by converting toxicity relative to 2,3,7,8-TCDD (Van den Berg et al. 1998). Low concentrations of few congeners in this study limited the utility of TEF for comparison. Total PCBs detected in this study did not exceed thresholds considered to be harmful to birds (Hoffman et al. 1996).

Organochlorine concentrations (geometric mean) in this study were generally lower than concentrations previously found in Aleutian avifauna (White and Risebrough 1977; Estes et al. 1997; Anthony et al. 1999; Crayton 2000; Stout and Trust 2002). This may be due to biomagnification, as the majority of contaminant research in the Aleutian Islands has been conducted on avian predators that are trophically elevated with respect to cormorants and rock sandpipers. Differing tissue matrices may also explain the lower concentrations detected in this study. I analyzed organochlorines in muscle tissue, which has less lipid and therefore lower accumulated OCs than eggs, which were analyzed in other studies (Estes et al. 1997; Anthony et al. 1999). Sampling techniques may also be responsible for concentration differences among studies. Other studies documenting contaminants in Aleutian birds have been conducted opportunistically on carcasses and unhatched eggs (Estes et al. 1997; Anthony et al. 1999; Stout and Trust 2002). Sampling

of living birds is more representative of contaminant concentrations in birds throughout the entire archipelago and provides baseline data for future comparison.

Trace metal concentrations in cormorant livers were also below those previously found in Amchitka cormorants and Adak bald eagles (Crayton 2000; Stout and Trust 2002). However, two cormorants from Attu Island that were found near death had some of the highest mercury and cadmium levels detected among Attu cormorants during this study. Even so, the concentrations in these two birds were not above levels considered to be harmful to birds (Thompson 1996; Furness 1996) and their moribund condition may be the result of the cumulative effects of contaminants. Mean levels of pollutants for this study and others conducted on Aleutian birds have been generally below levels considered to be harmful (Hoffman et al. 1995; Blus et al. 1996). However, declines in reproductive success in bald eagles (Estes et al. 1997; Anthony et al. 1999) and elevated levels in some of the cormorants examined in this study indicate that contaminants are a problem in the Aleutian Islands.

Despite complex air and ocean currents, unknown point sources, and increasing ship traffic between the western United States and southeast Asia, I detected patterns consistent with long-range atmospheric transport at two trophic levels. Global distribution of contaminants in the atmosphere, unlike point sources, cannot be reduced through mitigation. Contaminants in this region not only have implications for wildlife, but they also have the potential to impact one of North America's largest fisheries. Contaminant concentrations in high-latitude species are likely to rise with increasing industrial growth and emissions in Asia. Continued monitoring of sentinel species in this

unique ecosystem is crucial as slowly degrading compounds accumulate through continued atmospheric transport and deposition in the North Pacific.

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CHAPTER THREE

LINKING MOBILE ANIMALS TO POPULATIONS OF ORIGIN USING MULTIPLE MARKER CLASSES AND BAYESIAN ASSIGNMENT

Abstract

Linking mobile animals to source populations using intrinsic markers has only been successful at broad scales. Often, differences among populations in commonly used markers are not sufficient to permit accurate assignment of individuals at the geographic scale of interest. Increasing the number of weak markers used to delineate populations to should lead to more accurate assignment of individuals to sources. In Bayesian tests, continuous and discrete markers can be pooled to determine the probability of individual membership to source populations. To test whether percentages of correctly assigned individuals increase when multiple marker classes are combined, I developed a Bayesian method of assignment for mixed-class datasets and used three datasets, two real-world and one theoretical, comprised of continuous (isotopic, morphometric, and blood chemistry) and discrete (allelic genetic) markers to test it. In the simulated dataset, percentage of correct assignments increased with the addition of continuous markers until an asymptote was reached with eight markers. The highest percentage of correct assignments was 84% and occurred when all marker classes were included in the analyses. Similar increases in correct assignments were seen in the real-world Swainson's warbler (*Limnothlypis swainsonii*) and river otter (*Lontra canadensis*) datasets. The percentage of individuals correctly assigned when all marker classes were

included in the analyses was 27% and 42% for the warblers and otters, respectively. The low percentage of correct assignments was not surprising, considering the overall lack of population distinctiveness in both datasets and the high variance in some of the continuous markers in the otter dataset. Although multiple marker datasets are rare, further testing and the addition of exclusion thresholds will lead to more accurate assignment of individuals. At present, this method enables the combination of mixed-class datasets that may be used to delineate populations at scales that are useful to both ecologists and wildlife managers.

Introduction

The ability to assign mobile individuals to populations or geographic regions of origin has broad applicability to wildlife management, ecology, and conservation biology. In New World migratory birds, for example, our understanding of seasonal movements has been largely limited to the species and subspecies levels (e.g., AOU 1957), despite the likelihood that many species are distributed in geographically distinct populations, at least on their breeding grounds. In addition to seasonal movements, understanding dispersal patterns in vertebrates is a key component for evaluating population dynamics (e.g., Hendrick 1996) and to developing successful management practices. However, efforts to understand dispersal patterns and associations of individuals between their breeding and non-breeding distributions have been largely unsuccessful due to the general lack of population-level markers in many mobile animals.

Stable isotopes (Hobson et al. 2001; Hobson and Wassenaar 1997; Rubenstein et al. 2002), morphological characters (Ramos and Warner 1980; Robins and Schnell 1971),

and genetic markers (Haig 1997; Kimura et al. 2002) have been used to assess geographic variation in species and to link breeding and wintering areas of migratory birds, but these intrinsic markers have only proven useful at scales much broader than are practical for population- and individual-level questions. Intrinsic markers are often too "fuzzy" (i.e., provide weak distinction across a species' geographic range) to confidently assign individuals to source populations, because the level of among-population differentiation necessary for assigning individuals to correct source populations may be low in one class of marker (Graves et al. 2002). However, combining several fuzzy markers and classes of fuzzy markers may provide the resolution necessary to permit accurate assignment of individuals to source populations.

Combining continuous data such as isotopic and morphometric data with ordinal data such as genetic microsatellite data is impractical with classical statistical methods (Lee 1997). Bayesian methods use information that is summarized as a probability distribution and thus have no assumptions regarding the structure of the data, enabling results of multiple experiments or data from different sources to be combined in analyses (Gelman et al. 1995; Lee 1997; see Appendix 3.1 for Bayes' theorem). Therefore, categorical data, such as allelic genetic data, can be combined with continuous data (e.g., stable isotopes and morphology) to form larger and potentially more robust datasets to distinguish among populations. Bayesian analyses also enable the inclusion of non-normally distributed data and individuals with missing values. Because Bayesian methods calculate a posterior probability distribution that can be interpreted as the probability of origin for each individual, Bayesian analyses directly answers the question

"what are the probabilities of membership between an individual and a group of sampled populations?" These advantages over classical statistical methods permit development of a Bayesian method to combine multiple markers classes for assigning individuals to source populations.

Here, I developed a Bayesian approach of assignment that combines marker classes using a code developed to run with the WinBUGS 1.4 software package (Spiegelhalter et al. 2003). A simulated dataset was used to test the methodology and assumptions and to aid in the development of this analytical code ("Animal Cracker," Appendix 3.2). The performance of this method was tested by comparing percentages of correct assignments generated with only one class of marker with percentages from analyses on all marker classes combined. I then tested the efficacy of this method on two real-world datasets. Using these three datasets, I demonstrate the utility of combining marker classes and of using Bayesian assignment tests to increase resolution of assignment among populations.

Materials and Methods

I used a Bayesian method similar to that developed by Rannala and Mountain (1997) but modified to include three marker classes (two continuous and one discrete) to generate posterior probabilities of population membership for each individual. An individual's posterior probability of membership for each population was calculated by determining the frequency distribution of all candidate populations with that individual removed from the dataset and then calculating the likelihood of that individual occurring in each population. The individual was assigned to the population with the highest

probability of membership. In this method, individuals being assigned were given an equal prior probability of belonging in each population.

Prior information (called priors) regarding population structure can be incorporated into Bayesian analyses. Although I knew the true source population for each individual and therefore had *a priori* expectations of the outcome, I used non-informative priors (e.g., P[θ] = uniform distribution, where all values are equally likely) on all distributions, because a major criticism of Bayesian methods has been the incorporation of prior knowledge into analyses (Dennis 1996). Incorporating non-informative priors allows the distribution of the data to dominate the analyses and removes a potential source of bias from the model (Lee 1997). All parameters were given multivariate normal distributions with a Wishart distribution for variances, thereby allowing the data to dominate the analyses. This code was run with the WinBUGS 1.4 software package (Spiegelhalter et al. 2003; available for free download from http://www.mrc-bsu.cam.ac.uk/bugs), and it is given in annotated form in Appendix 3.2.

I evaluated this method by testing it on a simulated dataset first, then testing it further on two real-world datasets to determine the percentage of individuals correctly assigned to their true population of origin (the true population of origin was known for all individuals). I also analyzed each marker class independently to determine the percentage of correct assignment for that class of marker. Therefore, I were able to determine whether multiple marker classes increased correct assignment of individuals in these datasets. Assignments were considered random if their posterior probability of assignment was □ 1/(number of populations).

The amount of differentiation among populations influences the ability to assign individuals to source populations (Cornuet et al. 1999). To provide an overall metric on population divergence I calculated average percent pairwise differences for continuous markers for each dataset. The average percent pairwise difference for the dataset is the average of pairwise differences among all continuous markers. The average percent pairwise difference for the *i*th marker = $(|(X_{ij} - X_{ij+1})|/X_i) \times 100$, where X_{ij} = average for the *i*th marker, *j*th population, and X_i = average for all individuals across all populations of the *i*th marker.

Simulated Data. I simulated a dataset (Appendices 3.3 and 3.4) that was based on real-world values and variables for continuous and categorical (allelic genetic) data for Swainson's warblers (*Limnothlypis swainsonii*; see below). The dataset comprised 10 populations of 20 individuals each and consisted of continuous (two "stable isotope" and eight "skeletal") and discrete (five "microsatellite") variables. Continuous variables were generated for each marker using normally distributed random numbers (Appendix 3.3). I used the overall mean for each marker from the Swainson's warbler dataset to determine the means in the simulated dataset. The 10 population means in the simulated data differed from the warbler mean by increasing increments of 2.5% and were randomly assigned among populations. The average standard deviation among warbler populations was determined for each marker and was used as the standard deviation for each population for the corresponding marker in the simulated datasets. Therefore, standard deviations were constant among populations for each marker. The average percent pairwise difference among continuous variables was 9.27%.

Microsatellite data (Appendix 3.4) were simulated based on microsatellite characteristics (e.g., fragment sizes and ranges) for five loci from Swainson's warblers (Winker et al. 1999). For the five loci the number of alleles were 8, 20, 19, 15, and 11. Alleles were distributed among individuals to achieve a low-to-moderate level of population structure ($F_{st} = 0.057$).

Real-world data. Swainson's warblers are morphologically undifferentiated Nearctic-Neotropic migrants with a continuous breeding range in the coastal plain of the southeastern United States but with geographically distinct wintering areas in the Caribbean (Cuba and Jamaica) and mainland Middle America (southeastern Mexico and Belize; Brown and Dickson 1994).

Genetic (microsatellite) and isotopic data ($\delta^{13}C$ and $\delta^{15}N$) from feathers grown on breeding grounds come from other studies conducted on this species (Graves and Rubenstein unpubl., Winker et al. 2003). Skeletal measurements (Appendix 3.5) were taken from specimens collected from the breeding grounds between 27 April – 31 May 1986 – 1996 at nine sites in the species' breeding range ($n = 120$). Measurements were taken for 12 characters (skull depth, coracoid length, scapula length, keel length, keel depth, femur length, tibiotarsus length, tarsometatarsus length, humerus length, radius length, ulna length, and carpometacarpus length) according to Robins and Schnell (1971) using digital calipers to the nearest 0.01mm (Appendix 3.5). An unequal number of individuals comprised nine populations with three different marker classes: microsatellite ($n = 6$), stable isotopes ($n = 2$), and skeletal characters ($n = 12$). The Swainson's warbler

dataset had an average percent pairwise difference of 3.1% among all continuous variables, and population structure in microsatellites was reported in Winker et al. (2003).

North American river otters (*Lontra canadensis*) are year-round residents of Prince William Sound, Alaska, where populations are separated by large bodies of water (Blundell et al. 2002; Bowyer et al. 2003). I used blood chemistry, genetic (microsatellites), and fur isotope data (Appendix 3.6) collected from 113 animals between 1996 and 1998 at seven sampling sites from other studies (Blundell et al. 2002; Bowyer et al. 2003). There were unequal numbers of individuals among populations, and the dataset was comprised of three different marker classes: microsatellite (n = 5), stable isotopes (n = 2), and blood chemistry (n = 8). The river otter dataset had an average percent pairwise difference of 23.0% among all continuous variables. However, percent pairwise difference in some blood chemistry variables drove these overall difference values, with an extreme pairwise difference of 63% for one marker. Genetic population structure was reported in Blundell et al. (2002).

Results

Simulated data. As expected, the percentage of correct assignments increased asymptotically with the number of continuous ("stable isotope" and "skeletal") markers included in the analyses (Fig. 3.1). Combining classes had an effect similar to adding additional markers. When marker classes with an equal number of markers in each class (n = 2) were analyzed, correct assignments increased with the addition of marker classes. One marker class (n = 2) correctly assigned 25%, two marker classes (n = 4) 49%, and three markers classes (n = 6) 63% of individuals correctly.

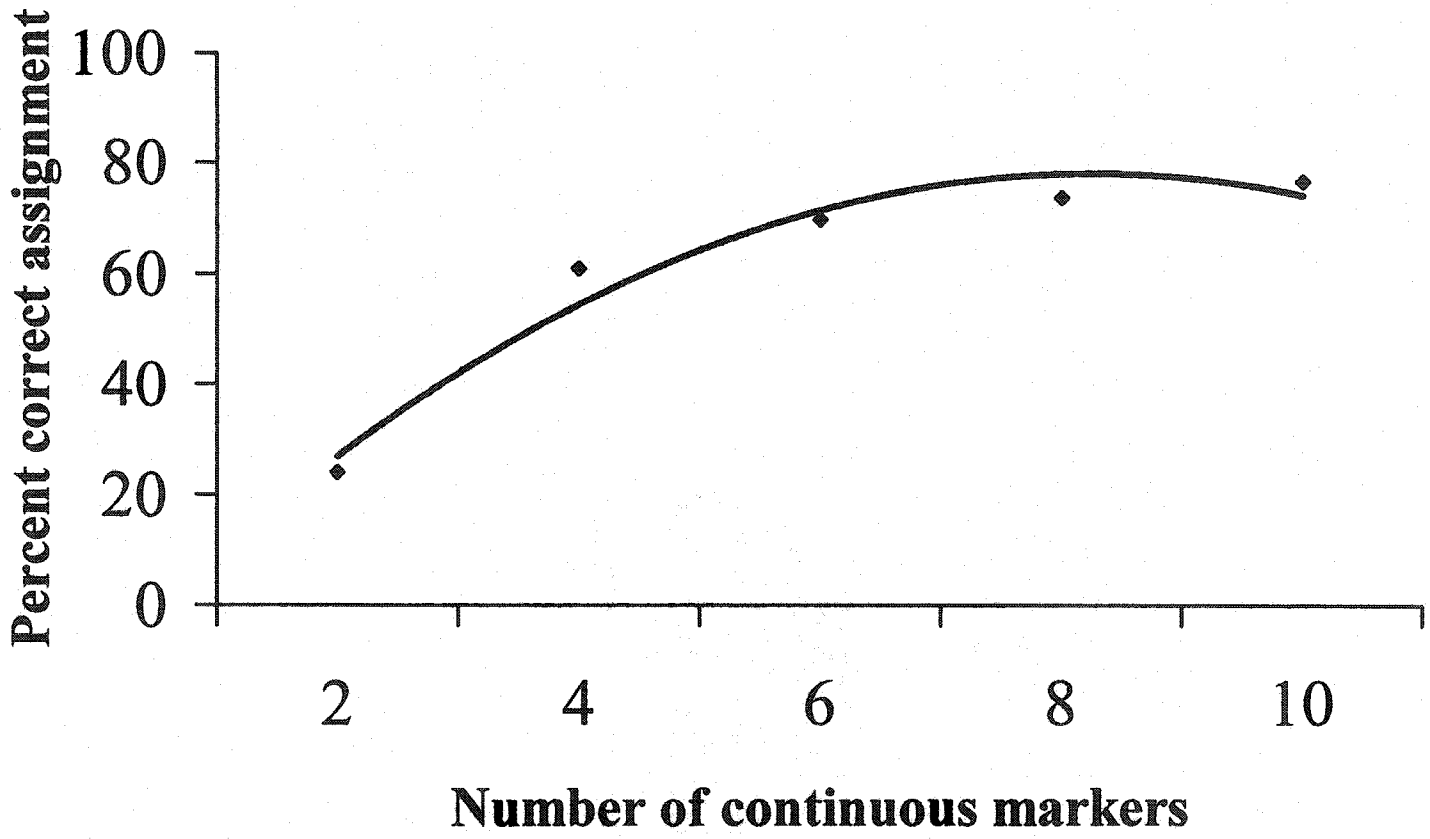

Figure 3.1. The percentage of correct assignment of individuals to populations of origin with increasing numbers of continuous ("stable isotope" and "skeletal") markers used in analysis. Percentage of correct assignments = $-5.21x^2 + 43.12x - 11$; $R^2 = 0.96$; $P < 0.05$.

Analyzed independently with the original number of markers in each class, "stable isotope", "skeletal", and "microsatellite" marker classes correctly assigned 25%, 74%, and 50% of individuals to their true source population, respectively (Table 3.1). Combining two marker classes increased the percentage of individuals correctly assigned over independent analyses in most cases, but the percentage of correct assignments did not increase with the combination of "microsatellite" and "skeletal" markers (Table 3.1). Combining all markers classes resulted in 84% of individuals correctly assigned to source populations (Table 3.1).

Swainson's Warblers. The skeletal markers correctly assigned 16%, stable isotopes 15%, and microsatellites 10% of individuals to their true source populations (Table 3.2). Only assignments based singly on microsatellites were not better than random assignment of 11%. In all cases, combining two marker classes in the analyses resulted in increased correct assignment of individuals (Table 3.2). Individual Swainson's warblers were assigned to their correct group of origin 27% of the time when all classes were included in the analysis (Table 3.2).

River Otters. Blood chemistry, stable isotopes, and microsatellites assigned 35%, 33%, and 25% of individuals to their true source populations, respectively (Table 3.3). When analyzed independently, marker classes assigned individuals more successfully than the random assignment probability of 14%. Combining two marker classes increased the percentage of individuals assigned over independent analyses in all cases (Table 3.3). Percentage of correct assignment was 42% when all three classes were included in the analysis (Table 3.3).

Table 3.1. Percentage of correct classification to group of origin for the simulated dataset for all marker classes and class combinations. Percentages ≤ 10% are not different from random assignments.

Markers						
Msat[1] ($n = 5$)[3]	Skeletal ($n = 8$)	SI[2] ($n = 2$)	Msat and Skeletal ($n = 13$)	Msat and SI[2] ($n = 7$)	Skeletal and SI ($n = 10$)	All markers ($n = 15$)
50	74	24.5	66	53	77	84

[1] Microsatellite.

[2] Stable isotope.

[3] Number of markers in class.

Table 3.2. Percentage of correct classification to group of origin for Swainson's warblers (*Limnothlypis swainsonii*) for all marker classes and class combinations. Percentages ≤ 11% are not different from random assignments.

Marker Classes						
Msat[1] ($n = 6$)[3]	Skeletal ($n = 12$)	SI[2] ($n = 2$)	Msat and Skeletal ($n = 18$)	Msat and SI ($n = 8$)	Skeletal and SI ($n = 14$)	All markers ($n = 20$)
10	16	15	16	23	22	27

[1] Microsatellite.

[2] Stable isotope.

[3] Number of markers in class.

Table 3.3. Percentage of correct classification to group of origin for river otters (*Lontra canadensis*) for all marker classes and class combinations. Percentages ≤ 14% are not different from random assignments.

Markers						
Msat[1] ($n = 5$)[4]	Blood[2] ($n = 8$)	SI[3] ($n = 2$)	Msat and Blood ($n = 13$)	Msat and SI ($n = 7$)	Blood and SI ($n = 10$)	All markers ($n = 15$)
28	30	33	33	39	35	42

[1] Microsatellite.

[2] Blood chemistry.

[3] Stable isotope.

[4] Number of markers in class.

Discussion

The analytical approach developed here provides increasing resolution with the addition of markers, but more importantly, with the addition of marker classes. Demonstrating that increased clarity of assignment among populations is achieved by combining multiple marker classes illustrates the potential breakthrough of this Bayesian method. The concept developed here is applicable to a wide range of biological disciplines, such as conservation, wildlife management, and ecology. At present, I provide a general analytical routine for assignment that can be easily modified for an increased number of markers and marker classes, and for incorporation of informative priors.

These analyses on continuous markers with a simulated dataset demonstrated that two markers with moderate among-populations differences resulted in a low percentage of assignment. This result should be noteworthy to stable isotope ecologists who are attempting to link breeding and wintering areas of birds using only the δD and $\delta^{13}C$ in feathers (Wassenaar and Hobson 2000; Rubenstein et al. 2002). However, results from these analyses demonstrated that when continuous "isotopic" markers were combined with other marker classes, the percentage of correct assignments increased substantially. These results would also likely increase with the addition of more regionally distinct isotopes.

Assignments to correct source populations were generally low in the two real-world datasets. These results are not surprising considering that previous studies attempting to detect differences among populations using single marker classes in

Swainson's warblers and river otters were not very successful (Blundell et al. 2002; Winker et al. 2003). Population structure was detected among Swainson's warbler populations using microsatellite data, but stochastic processes could not be ruled out to explain the low levels of structure observed (Winker et. al. 2003). The seven populations of river otters were marginally different genetically (Blundell et al. 2002) and isotopically but differences in blood chemistry were not detected (Bowyer et al. 2003).

The inclusion of informative priors is an advantage of Bayesian analyses and, when appropriate, would likely increase the posterior probability for some individuals in populations that have small average differences. For example, birds captured at some latitudes may have a higher probability of belonging to a particular population or group of populations, and this information can be incorporated into analyses. The inclusion of *a priori* knowledge is a valid approach to increasing posterior probabilities and can be added incrementally as new information regarding hypotheses becomes available (Ellison 1996), thereby providing a means to assess the effects that priors have on posterior assignment probabilities.

Incorporating exclusion thresholds into this method is an important next step to improve the applicability of this method. Without an exclusion criterion, this method assigned a high proportion of the individuals incorrectly. In the present method, populations with the highest probability of membership are designated as the source population, and therefore assignments occurred in individuals that had posterior probabilities of population membership that were no better than 27%. Because source populations for individuals in this study were known, I were able to assess the efficacy of

this method without an exclusion threshold. However, exclusion thresholds are necessary to confidently assign individuals when the true source population is unknown (Cornuet et al. 1999). Only by implementing assignment with exclusion in the next generation of tests can there be confidence that true source populations have been identified.

These results demonstrate that commonly used genetic and isotopic markers often lack the differentiation necessary among populations for accurate assignment of individuals at geographic levels of interest. I demonstrate the utility of combining marker classes to increase among-population resolution and that this can lead to improved assignment to source populations. This highlights the importance of obtaining multiple markers and marker classes in this type of research. I show that even with small difference among populations, assignment probabilities can be improved by adding markers and marker classes.

At present, this analytical routine ("Animal Cracker") can be used to assess the utility of multiple markers to delineate populations. It also has a broad range of applications where discriminant and logistic regression analyses are impractical due to non-normality and missing data. As pressures on wildlife populations increase, the ability to accurately link individuals to source populations and identify regions of diversity becomes critical. This and other methods that combine multiple marker classes have the potential to increase resolution among populations and assign individuals at scales of interest to managers.

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Appendix 3.1

Bayes' theorem (as used in Chapter 3)

Bayes' theorem states that the probability of the hypothesis (θ) given the data (*D*) is proportional to the product of the prior probability of the hypothesis (i.e., the expected outcome) and probability of the data given the hypothesis (likelihood):

$$P(\theta \mid D) \propto P(\theta) \times P(D \mid \theta). \quad (1)$$

In this study, the equation can be stated as "the probability of group membership between an individual and the sampled populations given the data is proportional to the product of the probability of random group membership and the probability of the data given the group membership between an individual and the sampled populations," and is written:

$$P(G \mid I, S, M) \propto P(G) \times P(I, S, M \mid G) \quad (2)$$

where G is group membership and I, S, and M are the "stable isotope", "skeletal", and "microsatellite" data. The probability of random group membership is a non-informative prior probability, where probability of membership to each group is equally likely.

Appendix 3.2

Annotated "ANIMAL CRACKER" code to run in WinBUGS 1.4

```
for (i in 1:N){
```

Where i is an integer and N is the sample size.

```
SI[i,1:2] ~ dmnorm(mux[group[i],1:2],taux[,])
```

SI has 2 variables with multivariate distributions (dmnorm) with the mean of each group mux and variance taux. Group[i] is the array of populations within the dataset.

```
Msat[i,1:10] ~ dmnorm(BigMusat[group[i],1:10], omega[,])
```

Msat has 10 variables with multivariate distributions (dmnorm) with the mean of each group BigMusat and variance omega. Group[i] is the array of populations within the datase. BigMusat is defined below.

```
Skel[i,1:8]~dmnorm(muskel[group[i],1:8], tauskel[,])
```

Skel has 8 variables with multivariate distributions (dmnorm) with the mean of each group muskel and variance tauskel. Group[i] is the array of populations within the dataset.

```
for (a in 1:10){
 BigMusat[a,1] <- musat[a,1]
 BigMusat[a,2] <- musat[a,1]
 BigMusat[a,3] <- musat[a,2]
 BigMusat[a,4] <- musat[a,2]
```

BigMusat[a,5] <- musat[a,3]

BigMusat[a,6] <- musat[a,3]

BigMusat[a,7] <- musat[a,4]

BigMusat[a,8] <- musat[a,4]

BigMusat[a,9] <- musat[a,5]

BigMusat[a,10] <- musat[a,5]

This array links the two alleles from each microsatellite locus so they are not analyzed as independent variables.

group[i] <- g[i]*(1-equals(QQ,i)) + newgroup*equals(QQ,i)

Removes the sample being assigned from the dataset before group distributions are calculated.

for (q in 1:10){

Defines q (an arbitrary letter chosen to represent the array) as having ten populations.

Assignment[q]<-equals(newgroup,q)

Assignment givese the posterior distribution (probability) of membership.

newgroup~dcat(p[])

Sets parameter newgroup as categorical.

for (j in 1:10){

Defines the size of the array (e.g., array has ten rows). Letter j is an arbitrary designation for the rows and must not be repeated in other arrays.

for (k in 1:2){

Defines the size of the array (e.g., array has two columns).

mux[j,k] ~ dnorm(0, 0.001)

Gives the mean mux a normal distribution with mean = 0 and 1/variance = 0.001 (1/variance is the convention used in Winbugs). Means and variances are initial values and true means and variances are calculated from the data, but they should be loosely representative of overall means for each marker class (thus variation among mux, musat, and muskel).

for (f in 1:10){

for (h in 1:5){

musat[f,h] ~ dnorm(200,0.001)

Same as above, but for mean musat.

for (a in 1:10){

for (b in 1:8){

muskel[a,b]~dnorm(10, 0.01)

Same as above, but for mean muskel.

for (z in 1:10){

p[z]<-1/10

Sets the prior probability of assignment to each new group as equal.

taux[1:2,1:2] ~ dwish(R[,],2)

Positive definite and symmetric distribution (matrix) around variance (required distribution for multivariate distributions). R is an arbitrary letter used to define the matrix in this array.

R[1,1] <- 1

Row 1, column 1 of the matrix = 1.

```
R[1,2] <- 0
```

Row 1, column 2 of the matrix = 0.

```
R[2,1] <- 0
```

Row 2, column 1 of the matrix = 0.

```
R[2,2] <- 1
```

Row 2, column 2 of the matrix = 1.

```
omega[1:10,1:10]~dwish(T[,],10)
```

Same as above under taux.

```
for (c in 1:5){
for (d in 1:5){
T[c,d]<-equals(c,d)
```

Shorthand for defining the matrix. Letter T is an arbitrary designation for the matrix.

```
tauskel[1:8,1:8]~dwish(S[,],8)
```

Same as above under taux.

```
for (q in 1:8){
for (r in 1:8){
S[q,r]<-equals(q,r)
```

Same as above.

```
END
```

Appendix 3.3

Mean (and variance) of continuous markers used in assignment test for simulated dataset

Population	Stable isotope 1	Stable isotope 2	Skeletal marker 1	Skeletal marker 2	Skeletal marker 3
1[1]	-21.79	7.06	13.96	7.53	14.40
	(0.61)	(3.86)	(0.18)	(0.03)	(0.19)
2	-25.41	6.67	13.67	8.14	14.26
	(0.25)	(3.43)	(0.09)	(0.03)	(0.16)
3	-23.64	6.23	17.11	6.63	14.91
	(0.38)	(5.85)	(0.17)	(0.08)	(0.08)
4	-23.10	6.19	16.66	6.60	15.93
	(0.52)	(1.99)	(0.08)	(0.06)	(0.08)
5	-25.11	6.36	14.86	6.75	15.67
	(0.48)	(2.37)	(0.17)	(0.05)	(0.12)

(continued)

Appendix 3.3

continued

Population	Stable isotope 1	Stable isotope 2	Skeletal marker 1	Skeletal marker 2	Skeletal marker 3
6	-24.78	7.22	16.40	7.97	16.48
	(0.28)	(4.69)	(0.13)	(0.07)	(0.19)
7	-20.75	7.36	15.27	7.82	13.85
	(0.20	(1.83)	(0.11)	(0.08)	(0.16)
8	-21.25	7.66	14.43	7.18	15.29
	(0.52)	(4.17)	(0.16)	(0.04)	(0.08)
9	-21.88	6.19	15.90	7.07	13.39
	(0.50)	(2.71)	(0.12)	(0.05)	(0.12)
10	-25.71	7.11	15.74	7.42	16.84
	(0.28)	(2.88)	(0.12)	(0.04)	(0.07)

(continued)

Appendix 3.3

continued

Population	Skeletal marker 4	Skeletal marker 5	Skeletal marker 6	Skeletal marker 7	Skeletal marker 8
1	23.92	15.41	16.15	19.35	11.11
	(0.85)	(0.09)	(0.16)	(0.08)	(0.04)
2	24.50	17.18	19.46	20.10	10.87
	(0.24)	(0.33)	(0.17)	(0.15)	(0.06)
3	28.09	16.39	18.53	20.68	10.05
	(0.37)	(0.17)	(0.22)	(0.12)	(0.04)
4	22.73	15.79	15.91	18.77	10.61
	(0.63)	(0.07)	(0.07)	(0.12)	(0.03)
5	23.39	14.86	18.20	18.23	9.30
	(0.24)	(0.11)	(0.27)	(0.22)	(0.06)

(continued)

Appendix 3.3

continued

Population	Skeletal marker 4	Skeletal marker 5	Skeletal marker 6	Skeletal marker 7	Skeletal marker 8
6	26.82	17.85	18.95	19.91	10.30
	(0.57)	(0.16)	(0.09)	(0.20)	(0.05)
7	26.63	16.74	19.96	21.73	9.57
	(0.41)	(0.17)	(0.21)	(0.18)	(0.06)
8	28.54	18.22	16.72	22.25	11.47
	(0.58)	(0.14)	(0.11)	(0.17)	(0.05)
9	25.70	17.41	17.58	21.23	9.80
	(0.41)	(0.28)	(0.15)	(0.17)	(0.03)
10	26.10	14.48	17.01	17.74	11.74
	(0.47)	(0.21)	(0.12)	(0.21)	(0.04)

[1] $n = 20$ individuals in all populations.

Appendix 3.4

Allelic distributions among five microsatellite loci used in the simulated dataset

pop

1 , 186186 263235 270268 225237 276286

1 , 184186 227241 262282 223221 290276

1 , 186188 231235 266278 223223 278290

1 , 186184 231243 274270 223237 290288

1 , 188188 259239 266268 223223 286290

1 , 198198 265237 276282 225223 294294

1 , 184186 229241 270264 219243 294286

1 , 186184 249235 276290 223223 278288

1 , 198198 227235 274264 221243 286284

1 , 186186 231241 270282 223221 278288

1 , 188186 227243 276266 221223 280284

1 , 188184 229239 272274 219239 278292

1 , 184188 231235 168282 219239 290282

1 , 186188 229237 266282 245221 288290

1 , 188184 231241 256288 245219 292292

1 , 186186 231239 260266 247221 280276

1 , 186186 231241 270266 221219 282288

1 , 184186 229239 276268 221221 286296

1 , 184186 229235 270274 241223 284288

1 , 192192 229239 262272 221221 296294

pop

2 , 184192 235235 274286 223227 276296

2 , 188190 233241 262282 219227 276292

2 , 186192 233241 266270 237227 276294

2 , 184194 233241 260276 235229 288294

2 , 186190 235241 270270 231231 286294

2 , 186194 235239 266272 219229 288290

2 , 186192 237239 270288 233231 292280

2 , 186192 235239 168288 225227 292296

2 , 186192 263237 262288 233229 292292

2 , 186192 233249 276264 223229 296280

2 , 186192 237239 262288 219229 296296

2 , 186198 237239 278284 219227 294292

2 , 186198 237241 262288 237227 294294

2 , 188194 233247 266286 223231 294296

2 , 188192 235241 278284 221245 292292

2 , 186192 233243 276280 221227 294292

2 , 188190 229227 280268 223245 278280

2 , 184194 233239 280266 223241 292294

2 , 184194 235261 280266 221229 286292

2 , 186192 237243 280286 221231 294294

pop

3 , 186196 241237 270286 227235 296282

3 , 186196 257255 260280 227235 292282

3 , 184194 241235 260286 229237 296288

3 , 188196 241235 272276 223237 292282

3 , 184194 239261 274288 229233 294286

3 , 188198 231237 260286 223235 292286

3 , 186196 229237 168270 227237 292288

3 , 186196 239237 274274 219247 294288

3 , 184196 243241 272288 221231 296284

3 , 184196 241235 280288 219237 296288

3 , 186194 239241 270266 221233 294290

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9 , 190192 245239 266268 225241 278286

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9 , 184186 245229 168282 223225 280288

9 , 192198 249257 168284 223245 280286

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10 , 190194 253251 256266 225235 292290

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10 , 194196 247257 258270 225229 280280

10 , 196194 245243 256266 225227 276286

10 , 186186 247265 260270 223223 282276

10 , 192188 247261 258268 227221 280276

10 , 188190 245243 260270 225233 280280

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Appendix 3.5

Mean (and variance) of continuous markers in Swainson's warblers (*Limnothlypis swainsonii*)

Population[1] (*n*)	$\delta^{13}C$	$\delta^{15}N$	Skull depth	Coracoid length	Scapula length	Keel length	Keel depth
VA	-23.57	6.54	10.62	16.10	18.32	17.48	7.51
(*n* = 8)	(0.56)	(1.29)	(0.06)	(0.09)	(0.08)	(0.66)	(0.09)
AR	-24.03	7.63	10.49	15.60	17.73	16.72	7.41
(*n* = 3)	(0.39)	(4.67)	(0.02)	(0.14)	(0.28)	(0.43)	(0.05)
FL	-23.22	7.69	10.54	15.68	17.84	16.95	7.38
(*n* = 14)	(0.59)	(8.00)	(0.04)	(0.12)	(0.19)	(0.44)	(0.10)
GA	-23.88	8.67	10.56	15.40	17.47	16.84	7.39
(*n* =19)	(0.49)	(7.49)	(0.06)	(0.14)	(0.29)	(0.30)	(0.05)
LA-1	-24.11	4.45	10.69	15.54	17.76	16.65	7.44
(*n* = 22)	(0.23)	(1.78)	(0.06)	(0.08)	(0.39)	(0.18)	(0.06)

(continued)

Appendix 3.5

continued

Population (*n*)	$\delta^{13}C$	$\delta^{15}N$	Skull depth	Coracoid length	Scapula length	Keel length	Keel depth
NC-1	-23.53	5.10	10.70	15.46	17.72	16.63	7.35
(*n* = 9)	(0.21)	(0.89)	(0.06)	(0.11)	(0.21)	(0.24)	(0.04)
SC-1	-24.17	6.27	10.51	15.80	18.05	17.08	7.52
(*n* = 11)	(0.45)	(2.73)	(0.02)	(0.21)	(0.25)	(0.60)	(0.08)
TX-2	-23.82	8.90	10.60	15.42	17.75	17.23	7.50
(*n* =18)	(0.38)	(4.20)	(0.05)	(0.13)	(0.45)	(0.41)	(0.08)
TX-1	-23.44	7.11	10.50	15.65	17.79	17.06	7.47
(*n* = 16)	(0.66)	(3.56)	(0.05)	(0.13)	(0.28)	(0.32)	(0.06)

(continued)

Appendix 3.5

continued

Population	Femur length	Tibiotarus length	Tarmetarus length	Humerus length	Radius length	Ulna length	Carpo[2] length
VA	15.46	26.46	18.45	17.05	18.46	20.65	10.85
	(0.16)	(0.25)	(0.17)	(0.09)	(0.11)	(0.09)	(0.02)
AR	15.22	25.79	18.15	16.54	17.99	20.14	10.58
	(0.07)	(0.37)	(0.15)	(0.11)	(0.18)	(0.18)	(0.11)
FL	15.30	25.93	18.04	16.61	17.99	20.17	10.36
	(0.15)	(0.50)	(0.22)	(0.07)	(0.19)	(0.17)	(0.14)
GA	15.26	25.28	18.02	16.53	17.95	20.14	10.55
	(0.08)	(0.36)	(0.13)	(0.11)	(0.13)	(0.09)	(0.01)
LA-1	15.33	25.96	18.04	16.70	18.09	20.38	10.60
	(0.17)	(0.21)	(0.27)	(0.10)	(0.06)	(0.11)	(0.03)

(continued)

Appendix 3.5

continued

Population	Femur length	Tibiotarus length	Tarmetarus length	Humerus length	Radius length	Ulna length	Carpo[1] length
NC-1	15.22	25.76	17.92	16.59	18.11	20.24	10.63
	(0.08)	(0.29)	(0.21)	(0.12)	(0.16)	(0.16)	(0.13)
SC-1	15.48	26.05	18.32	17.24	18.27	20.51	10.98
	(0.03)	(0.28)	(0.12)	(1.60)	(0.11)	(0.14)	(0.10)
TX-2	15.28	25.80	18.13	16.53	18.11	20.28	10.70
	(0.10)	(0.54)	(0.52)	(0.10)	(0.13)	(0.16)	(0.11)
TX-1	15.13	25.75	17.96	16.63	17.94	20.10	10.56
	(0.29)	(0.46)	(0.25)	(0.19)	(0.31)	(0.30)	(0.02)

[1] See Winker et al. (2003) for population names.

[2] Carpometacarpus.

Appendix 3.6

Mean (and variance) of continuous markers in river otters (*Lontra canadensis*)

Population[1] (*n*)	$\delta^{13}C$	$\delta^{15}N$	Glucose	BUN[2]	GGT
ROEI	-14.57	15.86	153	53	24
(*n* = 9)	(0.39)	(0.21)	(1666)	(122)	(3282)
ROEP	-15.12	15.68	152	42	26
(*n* = 11)	(0.69)	(0.34)	(3793)	(178)	(648)
ROHB	-14.66	15.74	148	47	33
(*n* = 32)	(1.18)	(0.37)	(4073)	(1486)	(6108)
ROJP	-15.11	16.04	107	39	54
(*n* = 42)	(1.49)	(0.27)	(1787)	(3719)	(11392)
RONI	-14.84	15.89	141	52	27
(*n* = 8)	(0.74)	(0.28)	(313)	(57)	(2953)
ROUI	-16.00	15.02	127	39	29
(*n* = 5)	(0.51)	(0.07)	(353)	(197)	(647)
ROWB	-15.61	15.43	122	49	29
(*n* = 8)	(3.15)	(0.82)	(275)	(150)	(894)

(continued)

Appendix 3.6

continued

Population (n)	ALKPHOS	LDH	SGOT	SGPT	Haptoglobin
ROEI	184	235	538	180	25
	(3282)	(26248)	(275840)	(9374)	(2716)
ROEP	128	178	295	191	22
	(648)	(5429)	(10654)	(2874)	(1414)
ROHB	167	191	375	140	34
	(6106)	(17672)	(107880)	(4647)	(2178)
ROJP	179	326	669	175	21
	(11392)	(52266)	(613167)	(10602)	(1866)
RONI	185	158	390	195	12
	(2952)	(8442)	(119490)	(8370)	(1081)
ROUI	145	154	371	178	48
	(647)	(7685)	(45175)	(6119)	(4461)
ROWB	152	324	619	158	7
	(894)	(45747)	(321519)	(6444)	(378)

[1,2] See Bowyer et al. (2003) for full names of populations and blood chemistry variables.

GENERAL CONCLUSION

My research attempting to link breeding and wintering areas in three intercontinental migrants with feather stable isotopes had mixed results. In two of the species significant differences were found between stable isotope ratios in feathers grown on breeding and wintering areas. However, this study provides a good example of three different migration systems in which commonly used intrinsic markers were not robust enough to permit accurate assignment of feathers to known continent of origin.

Long-range contaminant transport and point source hypotheses were directly tested, because stable isotope ratios in sentinel species enabled migration to be ruled out as a source of contaminants in the Aleutian Archipelago. While point sources for some pollutants were indicated, results from this study overarchingly supported the long-range transport hypothesis. These results supporting atmospheric transport have broad implications for wildlife populations and fisheries in the North Pacific, because contaminant concentrations in high-latitude species are likely to rise with increasing industrial growth and emissions in Asia. And, unlike point sources, contaminants that accumulate through global transport cannot be reduced through mitigation. This study therefore highlights the need for continued monitoring of sentinel species in this unique ecosystem.

In Chapter Three, individuals were assigned to populations using Bayesian methods developed to analyze datasets with discrete and continuous data. I demonstrated using two real-world and one theoretical example that increased assignment to true source populations can be accomplished by merging marker classes. In all

datasets, percentages of correct assignments were higher in merged datasets than percentages from single marker analyses. Because this method for combining marker classes is new, I provide the annotated code for the analyses in Appendix 3.2. Future work should include additional simulations to help refine this method and identify limits of applicability. The development of exclusion thresholds will make this method suitable to a wider range of disciplines.

Intrinsic markers may be used to link individuals to wintering and breeding areas, assess broad-scale population mixing throughout annual cycles, and delineate critical areas for conservation and management of migratory populations. Presently, no one class of marker provides sufficient resolution to answer most individual or population-level questions in complex systems. However, this research demonstrates the utility of intrinsic markers in ecological studies and outlines a diversity of questions these markers have the potential to answer.